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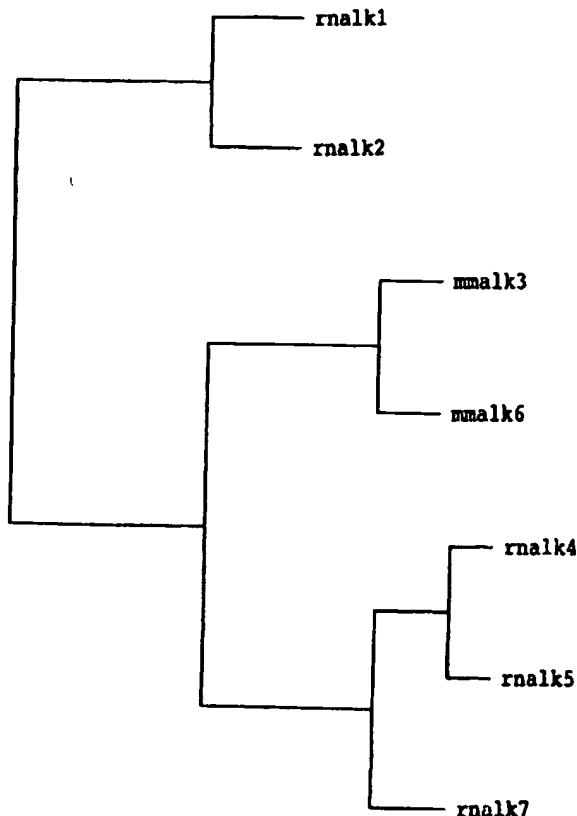
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(54) Title: ALK-7 (ACTIVIN LIKE KINASE), A SERINE THREONINE KINASE RECEPTOR

## (57) Abstract

The present invention relates, in general, to a novel serine threonine kinase receptor, ALK-7. In particular, the present invention relates to nucleic acid molecules coding for ALK-7; ALK-7 polypeptides; recombinant nucleic acid molecules; cells containing the recombinant nucleic acid molecules; antibodies having binding affinity specifically to ALK-7; hybridomas containing the antibodies; nucleic acid probes for the detection of ALK-7 nucleic acid; a method of detecting ALK-7 nucleic acid or polypeptide in a sample; and kits containing nucleic acid probes or antibodies. This invention further relates to bioassays using the nucleic acid sequence, receptor protein or antibodies of this invention to diagnose, assess, or prognose a mammal afflicted with neurodegenerative disease. Therapeutic uses for ALK-7 are also provided. This invention also relates to ligands, agonists, and antagonists of the ALK-7 receptor, and diagnostic and therapeutic uses thereof.



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## ALK-7 (Activin like kinase), SERINE THREONINE KINASE RECEPTOR

***Background of the Invention******Field of the Invention***

5 The present invention relates, in general, to a novel serine threonine kinase receptor, ALK-7. In particular, the present invention relates to nucleic acid molecules coding for ALK-7; purified ALK-7 polypeptides; recombinant nucleic acid molecules; cells containing the recombinant nucleic acid molecules; antibodies having binding affinity specifically to ALK-7; hybridomas containing the antibodies; nucleic acid probes for the detection of ALK-7 nucleic acid; a method of detecting ALK-7 nucleic acids or polypeptides in a sample; and kits containing nucleic acid probes or antibodies. This invention further relates to bioassays using the nucleic acid sequence, receptor protein or antibodies of this invention to diagnose, assess, or prognose a mammal afflicted with neurodegenerative disease. Therapeutic uses for ALK-7 are also provided. This invention also relates to ligands, agonists, and antagonists of the ALK-7 receptor, and diagnostic and therapeutic uses thereof.

***Background Information***

20 Serine threonine kinase receptors are a family of growth factor signal transducers (He *et al.* (1993) *Dev. Dyn.* 196:133-142). A series of serine threonine kinase receptors, activin receptor-like kinases 1-6 (ALK-1 to -6), have been previously identified (ten Dijke *et al.* (1993) *Oncogene* 8:2879-2887; Franzen *et al.* (1993) *Cell* 75:681-692; Ebner *et al.* (1993) *Science* 260:1344; Matsuzaki *et al.* (1993) *J. Biol. Chem.* 268:12719; He *et al.* (1993) *Dev. Dyn.* 196:133-142; Attisano *et al.* (1993) *Cell* 75:681-692; ten Dijke *et al.* (1994) *Science* 264:101-104; WO 94/11502 published May 26, 1994). The present invention provides a novel serine threonine kinase receptor, ALK-7.

### ***Summary of the Invention***

The invention provides an isolated nucleic acid molecule coding for a polypeptide comprising an amino acid sequence corresponding to a novel serine threonine kinase receptor, ALK-7.

5           The invention further provides a substantially pure polypeptide comprising an amino acid sequence corresponding to ALK-7.

The invention also provides a nucleic acid probe for the specific detection of the presence of ALK-7 in a sample.

10           The invention further provides a method of detecting ALK-7 nucleic acid in a sample.

The invention also provides a kit for detecting the presence of ALK-7 nucleic acid in a sample.

15           The invention further provides a recombinant nucleic acid molecule comprising, 5' to 3', a promoter effective to initiate transcription in a host cell and the above-described isolated nucleic acid molecule.

The invention also provides a recombinant nucleic acid molecule comprising a vector and the above-described isolated nucleic acid molecule.

20           The invention further provides a recombinant nucleic acid molecule comprising a sequence complimentary to an RNA sequence encoding an amino acid sequence corresponding to the above-described polypeptide.

The invention also provides a cell that contains the above-described recombinant nucleic acid molecule.

The invention further provides a non-human organism that contains the above-described recombinant nucleic acid molecule.

25           The invention also provides an antibody having binding affinity specifically to an ALK-7 polypeptide.

The invention further provides a method of detecting an ALK-7 polypeptide in a sample.

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The invention also provides a method of measuring the amount of ALK-7 in a sample.

The invention further provides a diagnostic kit comprising a first container means containing the above-described antibody, and a second container means containing a conjugate comprising a binding partner of the monoclonal antibody and a label.

The invention also provides a hybridoma which produces the above-described monoclonal antibody.

The invention further provides diagnostic methods for human disease, in particular neurodegenerative diseases, disorders, and injuries.

The invention also provides methods for therapeutic uses involving all or part of the nucleic acid sequence encoding ALK-7 and its corresponding protein.

The invention provides assays for the isolation of ligands, agonists and antagonists of the ALK-7 receptor and therapeutic uses for these molecules.

The invention also provides assays for the assessment and development of drugs capable of activating or suppressing the ALK-7 receptor and therapeutic uses for these drugs.

Further objects and advantages of the present invention will be clear from the description that follows.

### ***Definitions***

In the description that follows, a number of terms used in recombinant DNA (rDNA) technology are extensively utilized. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

***Isolated Nucleic Acid Molecule.*** An "isolated nucleic acid molecule", as is generally understood and used herein, refers to a polymer of nucleotides, and includes but should not be limited to DNA and RNA.

**DNA Segment.** A DNA segment, as is generally understood and used herein, refers to a molecule comprising a linear stretch of nucleotides wherein the nucleotides are present in a sequence that can encode, through the genetic code, a molecule comprising a linear sequence of amino acid residues that is referred to as a protein, a protein fragment or a polypeptide.

**Gene.** A DNA sequence related to a single polypeptide chain or protein, and as used herein includes the 5' and 3' untranslated ends. The polypeptide can be encoded by a full-length sequence or any portion of the coding sequence, so long as the functional activity of the protein is retained.

**Complementary DNA (cDNA).** Recombinant nucleic acid molecules synthesized by reverse transcription of messenger RNA ("mRNA").

**Structural Gene.** A DNA sequence that is transcribed into mRNA that is then translated into a sequence of amino acids characteristic of a specific polypeptide.

**Restriction Endonuclease.** A restriction endonuclease (also restriction enzyme) is an enzyme that has the capacity to recognize a specific base sequence (usually 4, 5, or 6 base pairs in length) in a DNA molecule, and to cleave the DNA molecule at every place where this sequence appears. For example, *EcoRI* recognizes the base sequence GAATTC/CTTAAG.

**Restriction Fragment.** The DNA molecules produced by digestion with a restriction endonuclease are referred to as restriction fragments. Any given genome can be digested by a particular restriction endonuclease into a discrete set of restriction fragments.

**Agarose Gel Electrophoresis.** To detect a polymorphism in the length of restriction fragments, an analytical method for fractionating double-stranded DNA molecules on the basis of size is required. The most commonly used technique (though not the only one) for achieving such a fractionation is agarose gel electrophoresis. The principle of this method is that DNA molecules migrate through the gel as though it were a sieve that retards the movement of the largest molecules to the greatest extent and the movement of the smallest molecules to

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the least extent. Note that the smaller the DNA fragment, the greater the mobility under electrophoresis in the agarose gel.

5 The DNA fragments fractionated by agarose gel electrophoresis can be visualized directly by a staining procedure if the number of fragments included in the pattern is small. The DNA fragments of genomes can be visualized successfully. However, most genomes, including the human genome, contain far too many DNA sequences to produce a simple pattern of restriction fragments. For example, the human genome is digested into approximately 1,000,000 different DNA fragments by *EcoRI*. In order to visualize a small subset of these  
10 fragments, a methodology referred to as the Southern hybridization procedure can be applied.

***Southern Transfer Procedure.*** The purpose of the Southern transfer procedure (also referred to as blotting) is to physically transfer DNA fractionated by agarose gel electrophoresis onto a nitrocellulose filter paper or another  
15 appropriate surface or method, while retaining the relative positions of DNA fragments resulting from the fractionation procedure. The methodology used to accomplish the transfer from agarose gel to nitrocellulose involves drawing the DNA from the gel into the nitrocellulose paper by capillary action.

***Nucleic Acid Hybridization.*** Nucleic acid hybridization depends on the  
20 principle that two single-stranded nucleic acid molecules that have complementary base sequences will reform the thermodynamically favored double-stranded structure if they are mixed under the proper conditions. The double-stranded structure will be formed between two complementary single-stranded nucleic acids even if one is immobilized on a nitrocellulose filter. In the  
25 Southern hybridization procedure, the latter situation occurs. As noted previously, the DNA of the individual to be tested is digested with a restriction endonuclease, fractionated by agarose gel electrophoresis, converted to the single-stranded form, and transferred to nitrocellulose paper, making it available for reannealing to the hybridization probe.

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**Hybridization Probe.** To visualize a particular DNA sequence in the Southern hybridization procedure, a labeled DNA molecule or hybridization probe is reacted to the fractionated DNA bound to the nitrocellulose filter. The areas on the filter that carry DNA sequences complementary to the labeled DNA probe become labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling are visualized. The hybridization probe is generally produced by molecular cloning of a specific DNA sequence.

**Oligonucleotide or Oligomer.** A molecule comprised of two or more deoxyribonucleotides or ribonucleotides, preferably more than three. Its exact size will depend on many factors, which in turn depend on the ultimate function or use of the oligonucleotide. An oligonucleotide can be derived synthetically or by cloning.

**Sequence Amplification.** A method for generating large amounts of a target sequence. In general, one or more amplification primers are annealed to a nucleic acid sequence. Using appropriate enzymes, sequences found adjacent to, or in between the primers are amplified.

**Amplification Primer.** An oligonucleotide which is capable of annealing adjacent to a target sequence and serving as an initiation point for DNA synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is initiated.

**Vector.** A plasmid or phage DNA or other DNA sequence into which DNA can be inserted to be cloned. The vector can replicate autonomously in a host cell, and can be further characterized by one or a small number of endonuclease recognition sites at which such DNA sequences can be cut in a determinable fashion and into which DNA can be inserted. The vector can further contain a marker suitable for use in the identification of cells transformed with the vector. Markers, for example, are tetracycline resistance or ampicillin resistance. The words "cloning vehicle" are sometimes used for "vector."

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**Expression.** Expression is the process by which a structural gene produces a polypeptide. It involves transcription of the gene into mRNA, and the translation of such mRNA into polypeptide(s).

5      **Expression Vector.** A vector or vehicle similar to a cloning vector but which is capable of expressing a gene which has been cloned into it, after transformation into a host. The cloned gene is usually placed under the control of (i.e., operably linked to) certain control sequences such as promoter sequences.

10      Expression control sequences will vary depending on whether the vector is designed to express the operably linked gene in a prokaryotic or eukaryotic host and can additionally contain transcriptional elements such as enhancer elements, termination sequences, tissue-specificity elements, and/or translational initiation and termination sites.

15      **Functional Derivative.** A "functional derivative" of a sequence, either protein or nucleic acid, is a molecule that possesses a biological activity (either functional or structural) that is substantially similar to a biological activity of the protein or nucleic acid sequence. A functional derivative of a protein can contain post-translational modifications such as covalently linked carbohydrate, depending on the necessity of such modifications for the performance of a specific function. The term "functional derivative" is intended to include the  
20      "fragments," "segments," "variants," "analogs," or "chemical derivatives" of a molecule.

25      As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties can improve the molecule's solubility, absorption, biological half life, and the like. The moieties can alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, and the like. Moieties capable of mediating such effects are disclosed in *Remington's Pharmaceutical Sciences* (1980). Procedures for coupling such moieties to a molecule are well known in the art.

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**Variant.** A "variant" of a protein or nucleic acid is meant to refer to a molecule substantially similar in structure and biological activity to either the protein or nucleic acid. Thus, provided that two molecules possess a common activity and can substitute for each other, they are considered variants as that term is used herein even if the composition or secondary, tertiary, or quaternary structure of one of the molecules is not identical to that found in the other, or if the amino acid or nucleotide sequence is not identical.

**Allele.** An "allele" is an alternative form of a gene occupying a given locus on the chromosome.

**Mutation.** A "mutation" is any detectable change in the genetic material which can be transmitted to daughter cells and possibly even to succeeding generations giving rise to mutant cells or mutant individuals. If the descendants of a mutant cell give rise only to somatic cells in multicellular organisms, a mutant spot or area of cells arises. Mutations in the germ line of sexually reproducing organisms can be transmitted by the gametes to the next generation resulting in an individual with the new mutant condition in both its somatic and germ cells. A mutation can be any (or a combination of) detectable, unnatural change affecting the chemical or physical constitution, mutability, replication, phenotypic function, or recombination of one or more deoxyribonucleotides; nucleotides can be added, deleted, substituted for, inverted, or transposed to new positions with and without inversion. Mutations can occur spontaneously and can be induced experimentally by application of mutagens. A mutant variation of a nucleic acid molecule results from a mutation. A mutant polypeptide can result from a mutant nucleic acid molecule.

**Species.** A "species" is a group of actually or potentially interbreeding natural populations. A species variation within a nucleic acid molecule or protein is a change in the nucleic acid or amino acid sequence that occurs among species and can be determined by DNA sequencing of the molecule in question.

**Substantially Pure.** A "substantially pure" protein or nucleic acid is a protein or nucleic acid preparation that is generally lacking in other cellular components.

5       **Ligand.** Ligand refers to any protein or proteins that can interact with the ALK-7 receptor binding domain. The ligand or ligands can be soluble or membrane bound. The ligand or ligands can be a naturally occurring protein, or synthetically or recombinantly produced. The ligand can also be a nonprotein molecule that acts as ligand when it interacts with the ALK-7 receptor binding domain. Interactions between the ligand and receptor binding domain include, 10 but are not limited to, any covalent or non-covalent interactions. The receptor binding domain is any region of the ALK-7 receptor molecule that interacts directly or indirectly with the ALK-7 ligand. Agonists and antagonists of ALK-7 that can interact with the ALK-7 receptor binding domain are ligands.

15       **Neurodegenerative disease.** The term neurodegenerative disease includes, but is not limited to, states in a mammal which can include chromosomal abnormalities, degenerative growth and developmental disorders, viral infections, bacterial infections, brain injuries, or neoplastic conditions. Examples of neurodegenerative diseases that can be diagnosed, assessed or treated by methods described in the present application include, but are not 20 limited to, Alzheimer's disease, epilepsy, schizophrenia. In a preferred embodiment diseases characterized by neurodegeneration in the limbic system are diagnosed, assessed or treated by methods disclosed in the present application. Examples of injuries to the nervous system include, but are not limited to, stroke and cerebral ischemia due to stroke or cardiac arrest. Also considered within this 25 definition is the treatment of injury to the nervous system. Further, neoplasms involving neuronal tissue can be diagnosed, assessed or therapeutically treated by methods suggested herein.

30       **Drug.** Drugs include, but are not limited to proteins, peptides, degenerate peptides, agents purified from conditioned cell medium, organic molecules, inorganic molecules, antibodies or oligonucleotides. Other candidate drugs

include analogs of the ALK-7 ligand or ligands. The drug can be naturally occurring or synthetically or recombinantly produced. One skilled in the art will understand that such drugs can be developed by the assays described below.

### ***Brief Description of the Drawings***

5                   ***Figure 1.*** Evolutionary relationships of the activin-like receptor kinase (ALK) family of serine-threonine kinase receptors. The relationship of ALK-7 to previously described receptors is shown. The amino acid sequences of the serine-threonine kinase domains of rat ALK-1, rat ALK-2, mouse ALK-3, rat  
10                   ALK-4, rat ALK-5, mouse ALK-6 and rat ALK-7 were aligned with the program PILEUP of the Genetics Computer Group of the University of Wisconsin. The alignment was then edited with LINEUP, and evolutionary relationships were calculated with DISTANCES using default algorithms. The plot was generated with GROWTREE.

***Figure 2.*** ALK-7 protein analysis.

15                   ***Figure 3.*** ALK-7 mRNA expression.

***Figure 4.*** ALK-7 mRNA expression in cerebellum.

***Figure 5.*** ALK-7 mRNA expression analyzed by hybridization *in situ*.

## Detailed Description of the Invention

For purposes of clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the following subsections:

- I. Isolated Nucleic Acid Molecules Coding for ALK-7 Polypeptides.
- II. Substantially Pure ALK-7 Polypeptides.
- III. A Nucleic Acid Probe for the Specific Detection of ALK-7.
- IV. A Method of Detecting The Presence of ALK-7 in a Sample.
- V. A Kit for Detecting the Presence of ALK-7 in a Sample.
- VI. DNA Constructs Comprising an ALK-7 Nucleic Acid Molecule and Cells Containing These Constructs.
- VII. An Antibody Having Binding Affinity to an ALK-7 Polypeptide and a Hybridoma Containing the Antibody.
- VIII. A Method of Detecting an ALK-7 Polypeptide in a Sample.
- IX. A Diagnostic Kit Comprising Antibodies to ALK-7.
- X. Diagnostic Screening and Treatment
- XI. Transgenic ALK-7 "Knock-Out" Mice

### *I. Isolated Nucleic Acid Molecules Coding for ALK-7 Polypeptides*

In one embodiment, the present invention relates to an isolated nucleic acid molecule coding for a polypeptide having an amino acid sequence corresponding to a novel serine threonine kinase receptor, ALK-7. In one preferred embodiment, the isolated nucleic acid molecule comprises an ALK-7 nucleotide sequence with greater than 70% similarity to the ALK-7 nucleotide sequence present in SEQ ID NO:1 (preferably greater than 80%; more preferably greater than 90%). In another preferred embodiment, the isolated nucleic acid molecule comprises the ALK-7 nucleotide sequence present in SEQ ID NO:1.

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In another embodiment, the isolated nucleic acid molecule encodes the ALK-7 amino acid sequence present in SEQ ID NO:2.

Also included within the scope of this invention are the functional equivalents of the herein-described isolated nucleic acid molecules and derivatives thereof. For example, the nucleic acid sequences depicted in SEQ ID NO:1 can be altered by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as depicted in SEQ ID NO:2 can be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of ALK-7 nucleic acid depicted in SEQ ID NO:1 which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change.

Such functional alterations of a given nucleic acid sequence afford an opportunity to promote secretion and/or processing of heterologous proteins encoded by foreign nucleic acid sequences fused thereto. All variations of the nucleotide sequence of the ALK-7 gene and fragments thereof permitted by the genetic code are, therefore, included in this invention.

In addition, the nucleic acid sequence can comprise a nucleotide sequence which results from the addition, deletion or substitution of at least one nucleotide to the 5'-end and/or the 3'-end of the nucleic acid formula shown in SEQ ID NO:1 or a derivative thereof. Any nucleotide or polynucleotide can be used in this regard, provided that its addition, deletion or substitution does not alter the amino acid sequence of SEQ ID NO:2 which is encoded by the nucleotide sequence. Moreover, the nucleic acid molecule of the present invention can, as necessary, have restriction endonuclease recognition sites added to its 5'-end and/or 3'-end.

Further, it is possible to delete codons or to substitute one or more codons by codons other than degenerate codons to produce a structurally modified polypeptide, but one which has substantially the same utility or activity of the

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polypeptide produced by the unmodified nucleic acid molecule. As recognized in the art, the two polypeptides are functionally equivalent, as are the two nucleic acid molecules which give rise to their production, even though the differences between the nucleic acid molecules are not related to degeneracy of the genetic code.

**A. Isolation of Nucleic Acid**

In one aspect of the present invention, isolated nucleic acid molecules coding for polypeptides having amino acid sequences corresponding to ALK-7 are provided. In particular, the nucleic acid molecule can be isolated from a biological sample containing human RNA or DNA.

The nucleic acid molecule can be isolated from a biological sample containing human RNA using the techniques of cDNA cloning and subtractive hybridization. The nucleic acid molecule can also be isolated from a cDNA library using a homologous probe.

The nucleic acid molecule can be isolated from a biological sample containing human genomic DNA or from a genomic library. Suitable biological samples include, but are not limited to, blood, semen and tissue. The method of obtaining the biological sample will vary depending upon the nature of the sample.

One skilled in the art will realize that the human genome can be subject to slight allelic variations between individuals. Therefore, the isolated nucleic acid molecule is also intended to include allelic variations, so long as the sequence is a functional derivative of the ALK-7 gene. When an ALK-7 allele does not encode the identical sequence to that found in SEQ ID NO:1, it can be isolated and identified as ALK-7 using the same techniques used herein, and especially PCR techniques to amplify the appropriate gene with primers based on the sequences disclosed herein.

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One skilled in the art will realize that organisms other than rats will also contain ALK-7 genes (for example, eukaryotes; more specifically, mammals, birds, fish, and plants; more specifically, humans, gorillas, rhesus monkeys, and chimpanzees; preferably, human ALK-7). The invention is intended to include, but not be limited to, ALK-7 nucleic acid molecules isolated from the above-described organisms.

### ***B. Synthesis of Nucleic Acid***

Isolated nucleic acid molecules of the present invention are also meant to include those chemically synthesized. For example, a nucleic acid molecule with the nucleotide sequence which codes for the expression product of an ALK-7 gene can be designed and, if necessary, divided into appropriate smaller fragments. Then an oligomer which corresponds to the nucleic acid molecule, or to each of the divided fragments, can be synthesized. Such synthetic oligonucleotides can be prepared, for example, by the triester method of Matteucci *et al.*, *J. Am. Chem. Soc.* 103:3185-3191 (1981) or by using an automated DNA synthesizer.

An oligonucleotide can be derived synthetically or by cloning. If necessary, the 5'-ends of the oligomers can be phosphorylated using T4 polynucleotide kinase. Kinasing of single strands prior to annealing or for labeling can be achieved using an excess of the enzyme. If kinasing is for the labeling of probe, the ATP can contain high specific activity radioisotopes. Then, the DNA oligomer can be subjected to annealing and ligation with T4 ligase or the like.

### ***II. Substantially Pure ALK-7 Polypeptides***

In another embodiment, the present invention relates to a substantially pure polypeptide having an amino acid sequence corresponding to ALK-7, or a

functional derivative thereof. In a preferred embodiment, the polypeptide has the amino acid sequence set forth in SEQ ID NO:2, or mutant or species variation thereof, or at least 70% identity or at least 85% similarity thereof (preferably, at least 90% identity or at least 95% similarity thereof), or at least 43 contiguous amino acids thereof (preferably, at least 50 or 100 contiguous amino acids thereof).

Amino acid sequence variants of ALK-7 can be prepared by mutations in the DNA. Such variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence shown in SEQ ID NO:2. Any combination of deletion, insertion, and substitution can also be made to arrive at the final construct, provided that the final construct possesses the desired activity. Obviously, the mutations that will be made in the DNA encoding the variant must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure.

While the site for introducing an amino acid sequence variation is predetermined, the mutation *per se* need not be predetermined. For example, to optimize the performance of a mutation at a given site, random mutagenesis can be conducted at the target codon or region and the expressed ALK-7 variants screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example, site-specific mutagenesis.

Preparation of an ALK-7 variant in accordance herewith is preferably achieved by site-specific mutagenesis of DNA that encodes an earlier prepared variant or a nonvariant version of the protein. Site-specific mutagenesis allows the production of ALK-7 variants through the use of specific oligonucleotide sequences that encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 20 to 25 nucleotides in length is preferred, with about 5 to 10 residues on both sides of the

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junction of the sequence being altered. In general, the technique of site-specific mutagenesis is well known in the art, as exemplified by publications such as Adelman *et al.*, *DNA* 2:183 (1983).

5 As will be appreciated, the site-specific mutagenesis technique typically employs a phage vector that exists in both a single-stranded and double-stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage, for example, as disclosed by Messing *et al.*, *Third Cleveland Symposium on Macromolecules and Recombinant DNA*, Editor A. Walton, Elsevier, Amsterdam (1981). These phage are readily commercially available  
10 and their use is generally well known to those skilled in the art. Alternatively, plasmid vectors that contain a single-stranded phage origin of replication (Vieira *et al.*, *Meth. Enzymol.* 153:3 (1987)) can be employed to obtain single-stranded DNA.

In general, site-directed mutagenesis in accordance herewith is performed  
15 by first obtaining a single-stranded vector that includes within its sequence a DNA sequence that encodes the relevant protein. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically, for example, by the method of Crea *et al.*, *Proc. Natl. Acad. Sci. (USA)* 75:5765 (1978). This primer is then annealed with the single-stranded protein-sequence-  
20 containing vector, and subjected to DNA-polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, to complete the synthesis of the mutation-bearing strand. Thus, a mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells and clones are selected that include recombinant vectors bearing the mutated  
25 sequence arrangement.

After such a clone is selected, the mutated protein region can be removed and placed in an appropriate vector for protein production, generally an expression vector of the type that can be employed for transformation of an appropriate host.

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Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably 1 to 10 residues, and typically are contiguous.

Amino acid sequence insertions include amino and/or carboxyl-terminal fusions of from one residue to polypeptides of essentially unrestricted length, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions (i.e., insertions within the complete ALK-7 sequence) can range generally from about 1 to 10 residues, more preferably 1 to 5.

The third group of variants are those in which at least one amino acid residue in the ALK-7 molecule, and preferably, only one, has been removed and a different residue inserted in its place. Such substitutions preferably are made in accordance with the following Table 1 when it is desired to modulate finely the characteristics of ALK-7.

TABLE 1

	<u>Original Residue</u>	<u>Exemplary Substitutions</u>
15	Ala	gly; ser
	Arg	lys
	Asn	gln; his
	Asp	glu
	Cys	ser
20	Gln	asn
	Glu	asp
	Gly	ala; pro
	His	asn; gln
	Ile	leu; val
25	Leu	ile; val
	Lys	arg; gln; glu
	Met	leu; tyr; ile
	Phe	met; leu; tyr
	Ser	thr
30	Thr	ser
	Trp	tyr
	Tyr	trp; phe
	Val	ile; leu

Substantial changes in functional or immunological identity are made by selecting substitutions that are less conservative than those in Table 1, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions that in general are expected to those in which (a) glycine and/or proline is substituted by another amino acid or is deleted or inserted; (b) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; (c) a cysteine residue is substituted for (or by) any other residue; (d) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) a residue having an electronegative charge, e.g., glutamyl or aspartyl; or (e) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having such a side chain, e.g., glycine.

Some deletions and insertions, and substitutions are not expected to produce radical changes in the characteristics of ALK-7. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays. For example, a variant typically is made by site-specific mutagenesis of the native ALK-7 encoding-nucleic acid, expression of the variant nucleic acid in recombinant cell culture, and, optionally, purification from the cell culture, for example, by immunoaffinity adsorption on a column (to absorb the variant by binding it to at least one remaining immune epitope). The activity of the cell lysate or purified ALK-7 molecule variant is then screened in a suitable screening assay for the desired characteristic. For example, a change in the immunological character of the ALK-7 molecule, such as affinity for a given antibody, is measured by a competitive type immunoassay. Changes in immunomodulation activity are measured by the appropriate assay. Modifications of such protein properties as redox or thermal stability,

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hydrophobicity, susceptibility to proteolytic degradation or the tendency to aggregate with carriers or into multimers are assayed by methods well known to the ordinarily skilled artisan.

5 A variety of methodologies known in the art can be utilized to obtain the peptide of the present invention. In one embodiment, the peptide is purified from tissues or cells which naturally produce the peptide. Alternatively, the above-described isolated nucleic acid fragments could be used to expressed the ALK-7 protein in any organism. The samples of the present invention include cells, protein extracts or membrane extracts of cells, or biological fluids. The sample  
10 will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts used as the sample.

Any eukaryotic organism can be used as a source for the peptide of the invention, as long as the source organism naturally contains such a peptide. As used herein, "source organism" refers to the original organism from which the  
15 amino acid sequence of the subunit is derived, regardless of the organism the subunit is expressed in and ultimately isolated from.

One skilled in the art can readily follow known methods for isolating proteins in order to obtain the peptide free of natural contaminants. These include, but are not limited to: immunochromoto-graphy, size-exclusion chromatography, HPLC, ion-exchange chromato-graphy, and immuno-affinity chromatography.  
20

### ***III. A Nucleic Acid Probe for the Specific Detection of ALK-7***

25 In another embodiment, the present invention relates to a nucleic acid probe for the specific detection of the presence of ALK-7 in a sample comprising the above-described nucleic acid molecules or at least a fragment thereof which binds under stringent conditions to ALK-7 but not ALK-1 through ALK-6. The probe is designed such that it does not have 100% homology with a similarly located ALK probe.

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The nucleic acid probe can be used to probe an appropriate chromosomal or cDNA library by usual hybridization methods to obtain another nucleic acid molecule of the present invention. A chromosomal DNA or cDNA library can be prepared from appropriate cells according to recognized methods in the art (cf. *Molecular Cloning: A Laboratory Manual, second edition*, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989).

In the alternative, chemical synthesis is carried out in order to obtain nucleic acid probes having nucleotide sequences which correspond to N-terminal and C-terminal portions of the amino acid sequence of the ALK-7. Thus, the synthesized nucleic acid probes can be used as primers in a polymerase chain reaction (PCR) carried out in accordance with recognized PCR techniques, essentially according to *PCR Protocols, A Guide to Methods and Applications*, edited by Michael *et al.*, Academic Press, 1990, utilizing the appropriate chromosomal or cDNA library to obtain the fragment of the present invention.

One skilled in the art can readily design such probes based on the sequence disclosed herein using methods of computer alignment and sequence analysis known in the art (cf. *Molecular Cloning: A Laboratory Manual, second edition*, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989).

The hybridization probes of the present invention can be labeled by standard labeling techniques such as with a radiolabel, enzyme label, fluorescent label, biotin-avidin label, chemiluminescence, and the like. After hybridization, the probes can be visualized using known methods.

The nucleic acid probes of the present invention include RNA, as well as DNA probes, such probes being generated using techniques known in the art.

In one embodiment of the above described method, a nucleic acid probe is immobilized on a solid support. Examples of such solid supports include, but are not limited to, plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, and acrylic resins, such as polyacrylamide and latex

beads. Techniques for coupling nucleic acid probes to such solid supports are well known in the art.

The test samples suitable for nucleic acid probing methods of the present invention include, for example, cells or nucleic acid extracts of cells, or biological fluids. The sample used in the above-described methods will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts to be assayed. Methods for preparing nucleic acid extracts of cells are well known in the art and can be readily adapted in order to obtain a sample which is compatible with the method utilized.

#### **IV. A Method of Detecting The Presence of ALK-7 in a Sample**

In another embodiment, the present invention relates to a method of detecting the presence of ALK-7 in a sample comprising a) contacting the sample with the above-described nucleic acid probe, under conditions such that hybridization occurs, and b) detecting the presence of the probe bound to the nucleic acid molecule. One skilled in the art would select the nucleic acid probe according to techniques known in the art as described above. Samples to be tested include but should not be limited to RNA samples of human tissue.

ALK-7 has been found to be expressed in brain cells. Accordingly, ALK-7 probes can be used detect the presence of RNA from brain cells in a sample. Further, altered expression levels of ALK-7 RNA in an individual, as compared to normal levels, can indicate the presence of disease. The ALK-7 probes can further be used to assay cellular activity in general and specifically in brain tissue.

#### **V. A Kit for Detecting the Presence of ALK-7 in a Sample**

In another embodiment, the present invention relates to a kit for detecting the presence of ALK-7 in a sample comprising at least one container means having disposed therein the above-described nucleic acid probe. In a preferred

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embodiment, the kit further comprises other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound nucleic acid probe. Examples of detection reagents include, but are not limited to radiolabelled probes, enzymatic labeled probes (horse radish peroxidase, alkaline phosphatase), and affinity labeled probes (biotin, avidin, or streptavidin).

In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow the efficient transfer of reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the probe or primers used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, and the like), and containers which contain the reagents used to detect the hybridized probe, bound antibody, amplified product, or the like.

One skilled in the art will readily recognize that the nucleic acid probes described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

#### ***VI. DNA Constructs Comprising an ALK-7 Nucleic Acid Molecule and Cells Containing These Constructs***

In another embodiment, the present invention relates to a recombinant DNA molecule comprising, 5' to 3', a promoter effective to initiate transcription in a host cell and the above-described nucleic acid molecules. In another embodiment, the present invention relates to a recombinant DNA molecule comprising a vector and an above-described nucleic acid molecule.

In another embodiment, the present invention relates to a nucleic acid molecule comprising a transcriptional control region functional in a cell, a sequence complimentary to an RNA sequence encoding an amino acid sequence corresponding to the above-described polypeptide, and a transcriptional termination region functional in the cell.

Preferably, the above-described molecules are isolated and/or purified DNA molecules.

In another embodiment, the present invention relates to a cell or non-human organism that contains an above-described nucleic acid molecule.

In another embodiment, the peptide is purified from cells which have been altered to express the peptide.

As used herein, a cell is said to be "altered to express a desired peptide" when the cell, through genetic manipulation, is made to produce a protein which it normally does not produce or which the cell normally produces at low levels. One skilled in the art can readily adapt procedures for introducing and expressing either genomic, cDNA, or synthetic sequences into either eukaryotic or prokaryotic cells.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene sequence expression. The precise nature of the regulatory regions needed for gene sequence expression can vary from organism to organism, but shall in general include a promoter region which, in prokaryotes, contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal synthesis initiation. Such regions will normally include those 5'-non-coding sequences involved with

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initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like.

If desired, the non-coding region 3' to the sequence encoding an ALK-7 gene can be obtained by the above-described methods. This region can be retained for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA sequence encoding an ALK-7 gene, the transcriptional termination signals can be provided. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a 3' region functional in the host cell can be substituted.

Two DNA sequences (such as a promoter region sequence and an ALK-7 sequence) are said to be operably linked if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region sequence to direct the transcription of an ALK-7 gene sequence, or (3) interfere with the ability of the ALK-7 gene sequence to be transcribed by the promoter region sequence. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence.

The present invention encompasses the expression of the ALK-7 gene (or a functional derivative thereof) in either prokaryotic or eukaryotic cells. Prokaryotic hosts are, generally, the most efficient and convenient for the production of recombinant proteins and, therefore, are preferred for the expression of the ALK-7 gene.

Prokaryotes most frequently are represented by various strains of *E. coli*. However, other microbial strains can also be used, including other bacterial strains. In prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host can be used. Examples of suitable plasmid vectors include pBR322, pUC118, pUC119 and the like; suitable phage or bacteriophage vectors include  $\lambda$ gt10,  $\lambda$ gt11 and the like; and suitable virus vectors include pMAM-neo, pKRC and the like. Preferably,

the selected vector of the present invention has the capacity to replicate in the selected host cell.

Recognized prokaryotic hosts include bacteria such as *E. coli*, *Bacillus*, *Streptomyces*, *Pseudomonas*, *Salmonella*, *Serratia*, and the like. However, under such conditions, the peptide will not be glycosylated. The prokaryotic host must be compatible with the replicon and control sequences in the expression plasmid.

To express ALK-7 in a prokaryotic cell, it is necessary to operably link the ALK-7 sequence to a functional prokaryotic promoter. Such promoters can be either constitutive or, more preferably, regulatable (i.e., inducible or derepressible). Examples of constitutive promoters include the *int* promoter of bacteriophage  $\lambda$ , the *bla* promoter of the  $\beta$ -lactamase gene sequence of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene sequence of pBR325, and the like. Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage  $\lambda$  ( $P_L$  and  $P_R$ ), the *trp*, *recA*, *lacZ*, *lacI*, and *gal* promoters of *E. coli*, the  $\alpha$ -amylase (Ulmanen *et al.*, *J. Bacteriol.* 162:176-182 (1985)) and the  $\zeta$ -28-specific promoters of *B. subtilis* (Gilman *et al.*, *Gene sequence* 32:11-20 (1984)), the promoters of the bacteriophages of *Bacillus* (Gryczan, In: *The Molecular Biology of the Bacilli*, Academic Press, Inc., NY (1982)), and *Streptomyces* promoters (Ward *et al.*, *Mol. Gen. Genet.* 203:468-478 (1986)). Prokaryotic promoters are reviewed by Glick (*J. Ind. Microbiol.* 1:277-282 (1987)); Cenatiempo (*Biochimie* 68:505-516 (1986)); and Gottesman (*Ann. Rev. Genet.* 18:415-442 (1984)).

Proper expression in a prokaryotic cell also requires the presence of a ribosome binding site upstream of the gene sequence-encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold *et al.* (*Ann. Rev. Microbiol.* 35:365-404 (1981)).

The selection of control sequences, expression vectors, transformation methods, and the like, are dependent on the type of host cell used to express the gene. As used herein, "cell", "cell line", and "cell culture" can be used interchangeably and all such designations include progeny. Thus, the words

"transformants" or "transformed cells" include the primary subject cell and cultures derived therefrom, without regard to the number of transfers. It is also understood that all progeny can not be precisely identical in DNA content, due to deliberate or inadvertent mutations. However, as defined, mutant progeny have the same functionality as that of the originally transformed cell.

Host cells which can be used in the expression systems of the present invention are not strictly limited, provided that they are suitable for use in the expression of the ALK-7 peptide of interest. Suitable hosts include eukaryotic cells.

Preferred eukaryotic hosts include, for example, yeast, fungi, insect cells, mammalian cells either *in vivo*, or in tissue culture. Preferred mammalian cells include HeLa cells, cells of fibroblast origin such as VERO or CHO-K1, or cells of lymphoid origin and their derivatives.

In addition, plant cells are also available as hosts, and control sequences compatible with plant cells are available, such as the cauliflower mosaic virus 35S and 19S, and nopaline synthase promoter and polyadenylation signal sequences.

Another preferred host is an insect cell, for example *Drosophila* larvae. Using insect cells as hosts, the *Drosophila* alcohol dehydrogenase promoter can be used. Rubin, *Science* 240:1453-1459 (1988). Alternatively, baculovirus vectors can be engineered to express large amounts of ALK-7 in insects cells (Jasny, *Science* 238:1653 (1987); Miller *et al.*, In: *Genetic Engineering* (1986), Setlow, J.K., *et al.*, eds., *Plenum*, Vol. 8, pp. 277-297).

Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, cleavage) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in mammalian cells can be used to ensure

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"native" glycosylation of the heterologous ALK-7 protein. Furthermore, different vector/host expression systems can effect processing reactions such as proteolytic cleavages to different extents.

5 Any of a series of yeast gene sequence expression systems can be utilized which incorporate promoter and termination elements from the actively expressed gene sequences coding for glycolytic enzymes are produced in large quantities when yeast are grown in mediums rich in glucose. Known glycolytic gene sequences can also provide very efficient transcriptional control signals.

10 Yeast provides substantial advantages in that it can also carry out post-translational peptide modifications. A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammalian gene sequence products and secretes peptides bearing leader sequences (i.e., pre-peptides). For a mammalian  
15 host, several possible vector systems are available for the expression of ALK-7.

20 A wide variety of transcriptional and translational regulatory sequences can be employed, depending upon the nature of the host. The transcriptional and translational regulatory signals can be derived from viral sources, such as adenovirus, bovine papilloma virus, simian virus, or the like, where the regulatory signals are associated with a particular gene sequence which has a high level of expression. Alternatively, promoters from mammalian expression products, such as actin, collagen, myosin, and the like, can be employed. Transcriptional  
25 initiation regulatory signals can be selected which allow for repression or activation, so that expression of the gene sequences can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or are subject to chemical (such as metabolite) regulation.

As discussed above, expression of ALK-7 in eukaryotic hosts requires the use of eukaryotic regulatory regions. Such regions will, in general, include a

promoter region sufficient to direct the initiation of RNA synthesis. Preferred eukaryotic promoters include, for example, the promoter of the mouse metallothionein I gene sequence (Hamer *et al.*, *J. Mol. Appl. Gen.* 1:273-288 (1982)); the TK promoter of Herpes virus (McKnight, *Cell* 31:355-365 (1982)); the SV40 early promoter (Benoist *et al.*, *Nature (London)* 290:304-310 (1981)); the yeast *gal4* gene sequence promoter (Johnston *et al.*, *Proc. Natl. Acad. Sci. (USA)* 79:6971-6975 (1982); Silver *et al.*, *Proc. Natl. Acad. Sci. (USA)* 81:5951-5955 (1984)).

As is widely known, translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine. For this reason, it is preferable to ensure that the linkage between a eukaryotic promoter and a DNA sequence which encodes ALK-7 does not contain any intervening codons which are capable of encoding a methionine (i.e., AUG). The presence of such codons results either in a formation of a fusion protein (if the AUG codon is in the same reading frame as the ALK-7 coding sequence) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the ALK-7 coding sequence).

An ALK-7 nucleic acid molecule and an operably linked promoter can be introduced into a recipient prokaryotic or eukaryotic cell either as a non-replicating DNA (or RNA) molecule, which can either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the gene can occur through the transient expression of the introduced sequence. Alternatively, permanent expression can occur through the integration of the introduced DNA sequence into the host chromosome.

In one embodiment, a vector is employed which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker can provide for prototrophy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as

copper, or the like. The selectable marker gene sequence can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements can also be needed for optimal synthesis of single chain binding protein mRNA. These elements can include splice signals, as well as transcription promoters, enhancers, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama, *Molec. Cell. Biol.* 3:280 (1983).

In a preferred embodiment, the introduced nucleic acid molecule will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors can be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector can be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species. Preferred prokaryotic vectors include plasmids such as those capable of replication in *E. coli* (such as, for example, pBR322, ColE1, pSC101, pACYC 184,  $\pi$ VX. Such plasmids are, for example, disclosed by Sambrook (cf. *Molecular Cloning: A Laboratory Manual*, second edition, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989). *Bacillus* plasmids include pC194, pC221, pT127, and the like. Such plasmids are disclosed by Gryczan (In: *The Molecular Biology of the Bacilli*, Academic Press, NY (1982), pp. 307-329). Suitable *Streptomyces* plasmids include pIJ101 (Kendall *et al.*, *J. Bacteriol.* 169:4177-4183 (1987)), and streptomyces bacteriophages such as  $\phi$ C31 (Chater *et al.*, In: *Sixth International Symposium on Actinomycetales Biology*, Akademiai Kiado, Budapest, Hungary (1986), pp. 45-54). *Pseudomonas* plasmids are reviewed by John *et al.* (*Rev. Infect. Dis.* 8:693-704 (1986)), and Izaki (*Jpn. J. Bacteriol.* 33:729-742 (1978)).

Preferred eukaryotic plasmids include, for example, BPV, vaccinia, SV40, 2-micron circle, and the like, or their derivatives. Such plasmids are well known

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in the art (Botstein *et al.*, *Miami Wntr. Symp.* 19:265-274 (1982); Broach, In: *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470 (1981); Broach, *Cell* 28:203-204 (1982); Bollon *et al.*, *J. Clin. Hematol. Oncol.* 10:39-48 (1980);  
5 Maniatis, In: *Cell Biology: A Comprehensive Treatise*, Vol. 3, Gene Sequence Expression, Academic Press, NY, pp. 563-608 (1980)).

Once the vector or nucleic acid molecule containing the construct(s) has been prepared for expression, the DNA construct(s) can be introduced into an appropriate host cell by any of a variety of suitable means, i.e., transformation,  
10 transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate-precipitation, direct microinjection, and the like. After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene molecule(s) results in the production of ALK-7. This can take  
15 place in the transformed cells as such, or following the induction of these cells to differentiate (for example, by administration of bromodeoxyuracil to neuroblastoma cells or the like).

**VII. An Antibody Having Binding Affinity to an ALK-7 Polypeptide and a Hybridoma Containing the Antibody**

20 In another embodiment, the present invention relates to an antibody having binding affinity specifically to an ALK-7 polypeptide as described above or specifically to an ALK-7 polypeptide binding fragment thereof. An antibody binds specifically to an ALK-7 polypeptide or binding fragment thereof if it does not bind to ALK-1 to ALK-6. Those which bind selectively to ALK-7 would be  
25 chosen for use in methods which could include, but should not be limited to, the analysis of altered ALK-7 expression in tissue containing ALK-7.

The ALK-7 proteins of the present invention can be used in a variety of procedures and methods, such as for the generation of antibodies, for use in

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identifying pharmaceutical compositions, and for studying DNA/protein interaction.

The ALK-7 peptide of the present invention can be used to produce antibodies or hybridomas. One skilled in the art will recognize that if an antibody is desired, such a peptide would be generated as described herein and used as an immunogen.

The antibodies of the present invention include monoclonal and polyclonal antibodies, as well as fragments of these antibodies. The invention further includes single chain antibodies. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')<sub>2</sub> fragment; the Fab' fragments, Fab fragments, and Fv fragments.

Of special interest to the present invention are antibodies to ALK-7 which are produced in humans, or are "humanized" (i.e. non-immunogenic in a human) by recombinant or other technology. Humanized antibodies can be produced, for example by replacing an immunogenic portion of an antibody with a corresponding, but non-immunogenic portion (i.e. chimeric antibodies) (Robinson, R.R. *et al.*, International Patent Publication PCT/US86/02269; Akira, K. *et al.*, European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison, S.L. *et al.*, European Patent Application 173,494; Neuberger, M.S. *et al.*, PCT Application WO 86/01533; Cabilly, S. *et al.*, European Patent Application 125,023; Better, M. *et al.*, *Science* 240:1041-1043 (1988); Liu, A.Y. *et al.*, *Proc. Natl. Acad. Sci. USA* 84:3439-3443 (1987); Liu, A.Y. *et al.*, *J. Immunol.* 139:3521-3526 (1987); Sun, L.K. *et al.*, *Proc. Natl. Acad. Sci. USA* 84:214-218 (1987); Nishimura, Y. *et al.*, *Canc. Res.* 47:999-1005 (1987); Wood, C.R. *et al.*, *Nature* 314:446-449 (1985)); Shaw *et al.*, *J. Natl. Cancer Inst.* 80:1553-1559 (1988). General reviews of "humanized" chimeric antibodies are provided by Morrison, S.L. (*Science*, 229:1202-1207 (1985)) and by Oi, V.T. *et al.*, *BioTechniques* 4:214 (1986)). Suitable "humanized" antibodies can be alternatively produced by CDR or CEA

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substitution (Jones, P.T. *et al.*, *Nature* 321:552-525 (1986); Verhoeyan *et al.*, *Science* 239:1534 (1988); Beidler, C.B. *et al.*, *J. Immunol.* 141:4053-4060 (1988)).

5 In another embodiment, the present invention relates to a hybridoma which produces the above-described monoclonal antibody. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

10 In general, techniques for preparing monoclonal antibodies and hybridomas are well known in the art (Campbell, "*Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*," Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth *et al.*, *J. Immunol. Methods* 35:1-21 (1980)).

15 Any animal (mouse, rabbit, and the like) which is known to produce antibodies can be immunized with the selected polypeptide. Methods for immunization are well known in the art. Such methods include subcutaneous or interperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of polypeptide used for immunization will vary based on the animal which is immunized, the antigenicity of the polypeptide and the site of injection.

20 The polypeptide can be modified or administered in an adjuvant in order to increase the peptide antigenicity. Methods of increasing the antigenicity of a polypeptide are well known in the art. Such procedures include coupling the antigen with a heterologous protein (such as globulin or  $\beta$ -galactosidase) or through the inclusion of an adjuvant during immunization.

25 For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells.

30 Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay,

western blot analysis, or radioimmunoassay (Lutz *et al.*, *Exp. Cell Res.* 175:109-124 (1988)).

Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, *supra* (1984)).

For polyclonal antibodies, antibody containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures.

In another embodiment of the present invention, the above-described antibodies are detectably labeled. Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, and the like), enzymatic labels (such as horse radish peroxidase, alkaline phosphatase, and the like) fluorescent labels (such as FITC or rhodamine, and the like), paramagnetic atoms, and the like. Procedures for accomplishing such labeling are well-known in the art, for example, see (Sternberger *et al.*, *J. Histochem. Cytochem.* 18:315 (1970); Bayer *et al.*, *Meth. Enzym.* 62:308 (1979); Engval *et al.*, *Immunol.* 109:129 (1972); Goding, *J. Immunol. Meth.* 13:215 (1976)). The labeled antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays to identify cells or tissues which express a specific peptide.

In another embodiment of the present invention the above-described antibodies are immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir *et al.*, "*Handbook of Experimental Immunology*" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby *et al.*, *Meth. Enzym.* 34 Academic Press, N.Y. (1974)). The immobilized antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays as well as in immunochromatography.

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Furthermore, one skilled in the art can readily adapt currently available procedures, as well as the techniques, methods and kits disclosed above with regard to antibodies, to generate peptides capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby *et al.*, "Application of Synthetic Peptides: Antisense Peptides", In *Synthetic Peptides, A User's Guide*, W.H. Freeman, NY, pp. 289-307 (1992), and Kaspczak *et al.*, *Biochemistry* 28:9230-8 (1989).

Anti-peptide peptides can be generated in one of two fashions. First, the anti-peptide peptides can be generated by replacing the basic amino acid residues found in the ALK-7 peptide sequence with acidic residues, while maintaining hydrophobic and uncharged polar groups. For example, lysine, arginine, and/or histidine residues are replaced with aspartic acid or glutamic acid and glutamic acid residues are replaced by lysine, arginine or histidine.

#### ***VIII. A Method of Detecting an ALK-7 Polypeptide in a Sample***

In another embodiment, the present invention relates to a method of detecting an ALK-7 polypeptide in a sample, comprising: a) contacting the sample with an above-described antibody, under conditions such that immunocomplexes form, and b) detecting the presence of the antibody bound to the polypeptide. In detail, the methods comprise incubating a test sample with one or more of the antibodies of the present invention and assaying whether the antibody binds to the test sample. Altered levels of ALK-7 in a sample as compared to normal levels can indicate a specific disease.

Conditions for incubating an antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the antibody used in the assay. One skilled in the art will recognize that any one of the commonly available immunological assay formats (such as radioimmunoassays, enzyme-linked immunosorbent assays, diffusion based Ouchterlony, or rocket immunofluorescent assays) can

readily be adapted to employ the antibodies of the present invention. Examples of such assays can be found in Chard, *An Introduction to Radioimmunoassay and Related Techniques*, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock *et al.*, *Techniques in Immunocytochemistry*, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, *Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985).

The immunological assay test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is capable with the system utilized.

#### ***IX. A Diagnostic Kit Comprising Antibodies to ALK-7***

In another embodiment of the present invention, a kit is provided which contains all the necessary reagents to carry out the previously described methods of detection. The kit can comprise: i) a first container means containing an above-described antibody, and ii) second container means containing a conjugate comprising a binding partner of the antibody and a label. In another preferred embodiment, the kit further comprises one or more other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound antibodies. Examples of detection reagents include, but are not limited to, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the chromophoric, enzymatic, or antibody binding reagents

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which are capable of reacting with the labeled antibody. The compartmentalized kit can be as described above for nucleic acid probe kits.

One skilled in the art will readily recognize that the antibodies described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

#### ***X. Diagnostic Screening and Treatment***

It is to be understood that although the following discussion is specifically directed to human patients, the teachings are also applicable to any animal that expresses ALK-7.

The diagnostic and screening methods of the invention are especially useful for a patient suspected of being at risk for developing a disease associated with an altered expression level of ALK-7 based on family history, or a patient in which it is desired to diagnose an ALK-7-related disease.

According to the invention, presymptomatic screening of an individual in need of such screening is now possible using DNA encoding the ALK-7 protein of the invention. The screening method of the invention allows a presymptomatic diagnosis, including prenatal diagnosis, of the presence of a missing or aberrant ALK-7 gene in individuals, and thus an opinion concerning the likelihood that such individual would develop or has developed an ALK-7-associated disease. This is especially valuable for the identification of carriers of altered or missing ALK-7 genes, for example, from individuals with a family history of an ALK-7-associated disease. Early diagnosis is also desired to maximize appropriate timely intervention.

In one preferred embodiment of the method of screening, a tissue sample would be taken from such individual, and screened for (1) the presence of the "normal" ALK-7 gene; (2) the presence of ALK-7 mRNA and/or (3) the presence of ALK-7 protein. The normal human gene can be characterized based upon, for example, detection of restriction digestion patterns in "normal" versus the

5 patient's DNA, including RFLP analysis, using DNA probes prepared against the ALK-7 sequence (or a functional fragment thereof) taught in the invention. Similarly, ALK-7 mRNA can be characterized and compared to normal ALK-7 mRNA (a) levels and/or (b) size as found in a human population not at risk of  
10 developing ALK-7-associated disease using similar probes. Lastly, ALK-7 protein can be (a) detected and/or (b) quantitated using a biological assay for ALK-7 activity or using an immunological assay and ALK-7 antibodies. When assaying ALK-7 protein, the immunological assay is preferred for its speed. An (1) aberrant ALK-7 DNA size pattern, and/or (2) aberrant ALK-7 mRNA sizes  
15 or levels and/or (3) aberrant ALK-7 protein levels would indicate that the patient is at risk for developing an ALK-7-associated disease.

The screening and diagnostic methods of the invention do not require that the entire ALK-7 DNA coding sequence be used for the probe. Rather, it is only  
20 necessary to use a fragment or length of nucleic acid that is sufficient to detect the presence of the ALK-7 gene in a DNA preparation from a normal or affected individual, the absence of such gene, or an altered physical property of such gene (such as a change in electrophoretic migration pattern).

Prenatal diagnosis can be performed when desired, using any known  
25 method to obtain fetal cells, including amniocentesis, chorionic villous sampling (CVS), and fetoscopy. Prenatal chromosome analysis can be used to determine if the portion of the chromosome possessing the normal ALK-7 gene is present in a heterozygous state.

In the method of treating an ALK-7-associated disease in a patient in need  
30 of such treatment, functional ALK-7 DNA can be provided to the cells of such patient in a manner and amount that permits the expression of the ALK-7 protein provided by such gene, for a time and in a quantity sufficient to treat such patient. Many vector systems are known in the art to provide such delivery to human patients in need of a gene or protein missing from the cell. For example, retrovirus systems can be used, especially modified retrovirus systems and especially herpes simplex virus systems. Such methods are provided for, in, for

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example, the teachings of Breakefield, X.A. *et al.*, *The New Biologist* 3:203-218 (1991); Huang, Q. *et al.*, *Experimental Neurology* 115:303-316 (1992), WO93/03743 and WO90/09441. Delivery of a DNA sequence encoding a functional ALK-7 protein will effectively replace the missing or mutated ALK-7 gene of the invention.

In an alternative embodiment stem cell populations for either neuronal or glial cells can be genetically engineered to express a functional ALK-7 receptor. Such cells recombinantly expressing the ALK-7 receptor, can be transplanted to the diseased or injured region of the mammal's neurological system (*Neural Transplantation. A Practical Approach*, Donnet & Djorklund, eds., Oxford University Press, New York, NY (1992)). In yet another alternative embodiment, embryonic tissue or fetal neurons can be genetically engineered to express functional ALK-7 receptor and transplanted to the diseased or injured region of the mammal's limbic system. The feasibility of transplanting fetal dopamine neurons into Parkinsonian patients has been demonstrated. (Lindvall *et al.*, *Archives of Neurology* 46:615-631 (1989)).

Studies of the molecular interactions between ligands and their receptors showed that only the extracellular domain of the receptor is involved in the special physical interaction between the molecules (Riedel *et al.*, *Nature* 324:68-70 (1986); Riedel *et al.*, *EMBO J.* 8:2943-2945 (1989)). Thus, the extracellular domain of a receptor can be used as a probe to screen an expression cDNA library for the ALK-7 ligand or ligands. In one approach for detection of the receptor probe, placental alkaline phosphatase will be fused to the extracellular domain of a receptor, and positive clones will be detected by the presence of alkaline phosphatase activity.

An alternative approach to isolate the ALK-7 ligand is to utilize the findings that co-expression of a receptor and its ligand in the same cells results in uncontrolled proliferation and malignant transformation (Klein *et al.*, *Cell* 66:395-403 (1991); Gazit *et al.*, *Cell* 39:89-97 (1984)). A eukaryotic cDNA expression library can be transfected into cells expressing a receptor, and the

presence of a ligand will create an autocrine loop, resulting in a transformed phenotype. This approach has been successfully used by Miki *et al.*, *Science* 251:72-75 (1991), to isolate the receptor of the keratinocyte growth factor (KGF) using cells expressing KGF. In yet another alternative approach the ALK-7  
5 receptor protein can be expressed in a cell line or in *Xenopus* oocytes by the recombinant techniques described above and its ligand stimulated activation of tyrosine kinase activity, as detected by an anti-phosphotyrosine antibody (UBI, Hapauge, New York) can be used to assay and purify the ligand. For example, cells expressing the recombinant ALK-7 receptor can be exposed to mammalian  
10 brain extract. The brain extracts can be fractionated by chromatography and used to assay for the presence of the ligand activity. Once an activity is identified in a particular fraction, it can be further purified by conventional biochemical techniques.

In another alternative approach, the ALK-7 extracellular domain can be  
15 used to screen a random peptide library (Cull *et al.*, *Proc. Natl. Acad. Sci. USA* 89:1865-1869 (1992); Lam *et al.*, *Nature* 354:82-84 (1991)). Peptides isolated can be assayed for their ligand activity.

In another embodiment of this invention, the ALK-7 ligand is expressed  
20 as a recombinant gene in a cell, so that the cells can be transplanted into a mammal, preferably a human in need of gene therapy. To provide gene therapy to an individual, a genetic sequence which encodes for all or part of the ALK-7 ligand is inserted into a vector and introduced into a host cell. Examples of diseases that can be suitable for gene therapy include, but are not limited to, neurodegenerative diseases or disorders, Alzheimer's, schizophrenia, epilepsy,  
25 neoplasms and cancer. Examples of vectors that may be used in gene therapy include, but are not limited to, defective retroviral, adenoviral, or other viral vectors (Mulligan, R.C., *Science* 260:926-932 (1993)). The means by which the vector carrying the gene can be introduced into the cell include but is not limited to, microinjection, electroporation, transduction, or transfection using  
30 DEAE-Dextran, lipofection, calcium phosphate or other procedures known to one

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skilled in the art (*Molecular Cloning, A Laboratory Manual*, Sambrook *et al.*, eds., Cold Spring Harbor Press, Plainview, New York (1989)).

The ability of antagonists and agonists of ALK-7 to interfere or enhance the activity of ALK-7 can be evaluated with cells containing ALK-7. An assay  
5 for ALK-7 activity in cells can be used to determine the functionality of the ALK-7 protein in the presence of an agent which may act as antagonist or agonist, and thus, agents that interfere or enhance the activity of ALK-7 are identified.

The agents screened in the assays can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. These agents  
10 can be selected and screened 1) at random, 2) by a rational selection or 3) by design using for example, protein or ligand modeling techniques.

For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to or stimulate/block the activity of ALK-7 protein.

Alternatively, agents may be rationally selected or designed. As used  
15 herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the ALK-7 protein or known ligand.

In one embodiment, the present invention relates to a method of screening for an antagonist or agonist which stimulates or blocks the activity of ALK-7  
20 comprising:

- (a) incubating a cell expressing ALK-7 with an agent to be tested; and
- (b) assaying the cell for the activity of the ALK-7 protein by measuring the agents effect on ALK-7 binding of ALK-7 ligand.

Any cell may be used in the above assay so long as it expresses a  
25 functional form of ALK-7 protein and the ALK-7 activity can be measured. The preferred expression cells are eukaryotic cells or organisms. Such cells can be modified to contain DNA sequences encoding the ALK-7 protein using routine procedures known in the art. Alternatively, one skilled in the art can introduce mRNA encoding the ALK-7 protein directly into the cell.

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Using ALK-7 ligands (including antagonists and agonists as described above) the present invention further provides a method for modulating the activity of the ALK-7 protein in a cell. In general, agents (antagonists and agonists) which have been identified to block or stimulate the activity of ALK-7 can be formulated so that the agent can be contacted with a cell expressing an ALK-7 protein *in vivo*. The contacting of such a cell with such an agent results in the *in vivo* modulation of the activity of the ALK-7 proteins. So long as a formulation barrier or toxicity barrier does not exist, agents identified in the assays described above will be effective for *in vivo* use.

In another embodiment, the present invention relates to a method of administering ALK-7 or an ALK-7 ligand (including ALK-7 antagonists and agonists) to an animal (preferably, a mammal (specifically, a human)) in an amount sufficient to effect an altered level of ALK-7 in the animal. The administered ALK-7 or ALK-7 ligand could specifically effect ALK-7 associated functions. Further, since ALK-7 is expressed in brain tissue, administration of ALK-7 or ALK-7 ligand could be used to alter ALK-7 levels in the brain.

One skilled in the art will appreciate that the amounts to be administered for any particular treatment protocol can readily be determined. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter indications, if any, and other such variables, to be adjusted by the individual physician. Dosage can vary from .001 mg/kg to 50 mg/kg of ALK-7 or ALK-7 ligand, in one or more administrations daily, for one or several days. ALK-7 or ALK-7 ligand can be administered parenterally by injection or by gradual perfusion over time. It can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive

oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose and sodium chloride, lactated Ringer's, or fixed oils.

5 Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives can also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like. See, generally, *Remington's Pharmaceutical Science*, 16th Ed., Mack Eds. (1980).

10 In another embodiment, the present invention relates to a pharmaceutical composition comprising ALK-7 or ALK-7 ligand in an amount sufficient to alter ALK-7 associated activity, and a pharmaceutically acceptable diluent, carrier, or excipient. Appropriate concentrations and dosage unit sizes can be readily determined by one skilled in the art as described above (See, for example,

15 *Remington's Pharmaceutical Sciences* (16th ed., Osol, A., Ed., Mack, Easton PA (1980) and WO 91/19008).

## ***XI. Transgenic ALK-7 "Knock-Out" Mice***

### **Methods of Generating Transgenic Non-Human Animals**

The non-human animals of the invention comprise any animal having a

20 transgenic interruption or alteration of the endogenous *ALK-7* gene(s) (knock-out animals) and/or into the genome of which has been introduced one or more transgenes that direct the expression of human *ALK-7*.

Such non-human animals include vertebrates such as rodents, non-human primates, sheep, dog, cow, amphibians, reptiles, etc. Preferred non-human

25 animals are selected from non-human mammalian species of animals, most preferably, animals from the rodent family including rats and mice, most preferably mice.

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The transgenic animals of the invention are animals into which has been introduced by nonnatural means (i.e., by human manipulation), one or more genes that do not occur naturally in the animal, e.g., foreign genes, genetically engineered endogenous genes, etc. The nonnaturally introduced genes, known as transgenes, may be from the same or a different species as the animal but not naturally found in the animal in the configuration and/or at the chromosomal locus conferred by the transgene. Transgenes may comprise foreign DNA sequences, i.e., sequences not normally found in the genome of the host animal. Alternatively or additionally, transgenes may comprise endogenous DNA sequences that are abnormal in that they have been rearranged or mutated *in vitro* in order to alter the normal *in vivo* pattern of expression of the gene, or to alter or eliminate the biological activity of an endogenous gene product encoded by the gene. (Watson, J.D., *et al.*, in *Recombinant DNA*, 2d Ed., W.H. Freeman & Co., New York (1992), pages 255-272; Gordon, J.W., *Intl. Rev. Cytol.* 115:171-229 (1989); Jaenisch, R., *Science* 240:1468-1474 (1989); Rossant, J., *Neuron* 2:323-334 (1990)).

The transgenic non-human animals of the invention are produced by introducing transgenes into the germline of the non-human animal. Embryonic target cells at various developmental stages are used to introduce the transgenes of the invention. Different methods are used depending on the stage of development of the embryonic target cell(s).

1. Microinjection of zygotes is the preferred method for incorporating transgenes into animal genomes in the course of practicing the invention. A zygote, a fertilized ovum that has not undergone pronuclei fusion or subsequent cell division, is the preferred target cell for microinjection of transgenic DNA sequences. The murine male pronucleus reaches a size of approximately 20 micrometers in diameter, a feature which allows for the reproducible injection of 1-2 picoliters of a solution containing transgenic DNA sequences. The use of a zygote for introduction of transgenes has the advantage that, in most cases, the injected transgenic DNA sequences will be incorporated into the host animal's

genome before the first cell division (Brinster, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 82:4438-4442 (1985)). As a consequence, all cells of the resultant transgenic animals (founder animals) stably carry an incorporated transgene at a particular genetic locus, referred to as a transgenic allele. The transgenic allele demonstrates Mendelian inheritance: half of the offspring resulting from the cross of a transgenic animal with a non-transgenic animal will inherit the transgenic allele, in accordance with Mendel's rules of random assortment.

2. Viral integration can also be used to introduce the transgenes of the invention into an animal. The developing embryos are cultured *in vitro* to the developmental stage known as a blastocyst. At this time, the blastomeres may be infected with appropriate retroviruses (Jaenich, R., *Proc. Natl. Sci. (USA)* 73:1260-1264 (1976)). Infection of the blastomeres is enhanced by enzymatic removal of the zona pellucida (Hogan, *et al.*, in *Manipulating the Mouse Embryo*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1986)). Transgenes are introduced via viral vectors which are typically replication-defective but which remain competent for integration of viral-associated DNA sequences, including transgenic DNA sequences linked to such viral sequences, into the host animal's genome (Jahner, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 82:6927-6931 (1985); Van der Putten, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 82:6148-6152 (1985)). Transfection is easily and efficiently obtained by culture of blastomeres on a mono-layer of cells producing the transgene-containing viral vector (Van der Putten, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 82:6148-6152 (1985); Stewart, *et al.*, *EMBO Journal* 6:383-388 (1987)). Alternatively, infection may be performed at a later stage, such as a blastocoele (Jahner, D., *et al.*, *Nature* 298:623-628 (1982)). In any event, most transgenic founder animals produced by viral integration will be mosaics for the transgenic allele; that is, the transgene is incorporated into only a subset of all the cells that form the transgenic founder animal. Moreover, multiple viral integration events may occur in a single founder animal, generating multiple transgenic alleles which will segregate in future generations of offspring. Introduction of transgenes into germline cells by this

method is possible but probably occurs at a low frequency (Jahner, D., *et al.*, *Nature* 298:623-628 (1982)). However, once a transgene has been introduced into germline cells by this method, offspring may be produced in which the transgenic allele is present in all of the animal's cells, i.e., in both somatic and germline cells.

3. Embryonic stem (ES) cells can also serve as target cells for introduction of the transgenes of the invention into animals. ES cells are obtained from pre-implantation embryos that are cultured *in vitro* (Evans, M.J., *et al.*, *Nature* 292:154-156 (1981); Bradley, M. O., *et al.*, *Nature* 309:255-258 (1984); Gossler, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 83:9065-9069 (1986); Robertson *et al.*, *Nature* 322:445-448 (1986); Robertson, E.J., in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, E.J., ed., IRL Press, Oxford (1987), pages 71-112). ES cells, which are commercially available (from, e.g., Genome Systems, Inc., St. Louis, MO), can be transformed with one or more transgenes by established methods (Lovell-Badge, R.H., in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, E.J., ed., IRL Press, Oxford (1987), pages 153-182). Transformed ES cells can be combined with an animal blastocyst, whereafter the ES cells colonize the embryo and contribute to the germline of the resulting animal, which is a chimera (composed of cells derived from two or more animals) (Jaenisch, R., *Science* 240:1468-1474 (1988); Bradley, A., in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, E.J., ed., IRL Press, Oxford (1987), pages 113-151). Again, once a transgene has been introduced into germline cells by this method, offspring may be produced in which the transgenic allele is present in all of the animal's cells, i.e., in both somatic and germline cells.

However it occurs, the initial introduction of a transgene is a Lamarckian (non-Mendelian) event. However, the transgenes of the invention may be stably integrated into germ line cells and transmitted to offspring of the transgenic animal as Mendelian loci. Other transgenic techniques result in mosaic transgenic animals, in which some cells carry the transgenes and other cells do

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not. In mosaic transgenic animals in which germ line cells do not carry the transgenes, transmission of the transgenes to offspring does not occur. Nevertheless, mosaic transgenic animals are capable of demonstrating phenotypes associated with the transgenes.

5 Transgenes may be introduced into non-human animals in order to provide animal models for human diseases. Transgenes that result in such animal models include, e.g., transgenes that encode mutant gene products associated with an inborn error of metabolism in a human genetic disease and transgenes that encode a human factor required to confer susceptibility to a human pathogen (i.e.,  
10 a bacterium, virus, or other pathogenic microorganism) (Leder *et al.*, U.S. Patent 5,175,383 (Dec. 29, 1992); Kindt *et al.*, U.S. Patent 5,183,949 (Feb. 2, 1993); Small *et al.*, *Cell* 46:13-18 (1986); Hooper *et al.*, *Nature* 326:292-295 (1987); Stacey *et al.*, *Nature* 332:131-136 (1988); Windle *et al.*, *Nature* 343:665-669 (1990); Katz *et al.*, *Cell* 74:1089-1100 (1993)). Transgenically introduced  
15 mutations comprise null ("knock-out") alleles in which a DNA sequence encoding a selectable and/or detectable marker is substituted for a genetic sequence normally endogenous to a non-human animal. Resultant transgenic non-human animals that are predisposed to a disease, or in which the transgene causes a disease, may be used to identify compositions that induce the disease and  
20 to evaluate the pathogenic potential of compositions known or suspected to induce the disease (Berns, A.J.M., U.S. Patent 5,174,986 (Dec. 29, 1992)), or to evaluate compositions which may be used to treat the disease or ameliorate the symptoms thereof (Scott *et al.*, WO 94/12627 (1994)).

25 Offspring that have inherited the transgenes of the invention are distinguished from littermates that have not inherited transgenes by analysis of genetic material from the offspring for the presence of biomolecules that comprise unique sequences corresponding to sequences of, or encoded by, the transgenes of the invention. For example, biological fluids that contain polypeptides uniquely encoded by the selectable marker of the transgenes of the  
30 invention may be immunoassayed for the presence of the polypeptides. A more

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simple and reliable means of identifying transgenic offspring comprises obtaining a tissue sample from an extremity of an animal, e.g., a tail, and analyzing the sample for the presence of nucleic acid sequences corresponding to the DNA sequence of a unique portion or portions of the transgenes of the invention, such as the selectable marker thereof. The presence of such nucleic acid sequences may be determined by, e.g., hybridization ("Southern") analysis with DNA sequences corresponding to unique portions of the transgene, analysis of the products of PCR reactions using DNA sequences in a sample as substrates and oligonucleotides derived from the transgene's DNA sequence, etc.

The present invention is described in further detail in the following non-limiting examples.

### *Example 1* *Isolation of ALK-7*

A PCR fragment of ALK-7 was isolated using primer 5'-GCGGATCCGT(C/G/T)CG(A/C/T)GT(C/G/T)AA(A/G)AT(A/C/T)TT-3' (SEQ ID NO:3), derived from the motif VAVKIF (SEQ ID NO:4) with a BamHI restriction site and primer 5'-CGGAATTC(A/G/T)GG(A/G/T)GCCAT(A/G)TA-3' (SEQ ID NO:5), derived from the motif YMAPE (SEQ ID NO:6) with an EcoRI restriction site. PCR amplification was done for five cycles, each composed of 94°C (1 min), 50°C (1 min) and 72°C (1 min), followed by 35 cycles, each composed of 94°C (1 min), 55°C (1 min) and 72°C (1 min).

This PCR fragment was used to clone a full-length cDNA from a  $\lambda$ gt10 postnatal day 7 total rat-brain library, washing at high stringency (60°C, 0.1% SDS, 0.1 XSSC) (for general techniques, *See, Molecular Cloning: A Laboratory Manual, second edition*, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989). The full length ALK-7 cDNA clone was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 and was given the accession number ATCC 75945. The nucleotide

and amino acid sequences of the coding region of the ALK-7 clone is presented in SEQ ID NOS:1 and 2, respectively.

## ***Example 2***

### ***Isolation of an ALK-7 Ligand***

#### ***5 Screening of a cDNA Expression Library For ALK-7 Ligand Using an Extracellular Domain-Alkaline Phosphatase Fusion Protein As a Probe***

To construct a fusion protein between the extracellular domain of ALK-7 and the secreted placental alkaline phosphatase (SEAP), a vector named APtag-1, constructed by Flannagan & Leder, *Cell* 63:185-194 (1990), is used. APtag-1  
10 contains a set of restriction sites for the insertion of the region of the ALK-7 cDNA encoding the extracellular domain. Downstream of the insertion sites is the full length sequence of SEAP, which is fused to the upstream sequence.

To generate an ALK-7 receptor fusion protein, the 5' end of the ALK-7 cDNA sequence is inserted into APtag-1, including sequences encoding the ALK-  
15 7 secretion signal peptide and the entire extracellular domain, ending immediately before the first hydrophobic amino acid of the transmembrane region. The resulting plasmid encodes a fusion protein with the ALK-7 extracellular domain joined to a SEAP. The fusion protein is expressed from a Moloney Murine Leukemia virus LTR promoter. The fusion construct is transfected into NIH/3T3  
20 cells which have been shown to express high levels of an APtag-Kit fusion protein (Flannagan & Leder, *Cell* 63:185-194 (1990)). The fusion construct is cotransfected with a selectable marker plasmid pSV2neo, and selected with G418 (400-800 µg/ml). Neo-resistant colonies are grown in 96-well plates and screened for secretion of SEAP activity into the media. The fusion protein is  
25 concentrated, purified and used as a probe to screen a cDNA expression library from mammalian brain, preferably human.

Three types of positive clones are expected: (1) clones having background alkaline phosphatase activity; (2) clones which bind non-specifically to the fusion protein; and (3) clones encoding the ALK-7 ligand. Background phosphatase clones will be positive without the added probe in the presence of alkaline phosphatase substrates. To distinguish the specific from the non-specific interacting clones, extracts from bacteria expressing these clones are used to stimulate the kinase activity of ALK-7 in ALK-7 expressing NIH/3T3 cells. Only the ligand will be able to stimulate activation of ALK-7 kinase activity.

It is preferable to produce the receptor probe in NIH/3T3 cells rather than bacteria to receive proper glycosylation of the ALK-7 extracellular domain. It has however been demonstrated that glycosylation of growth factors is often not necessary for their activity. For example, M-CSF (Metcalf, *Blood* 67:257-267 (1986) and NGF (available from Boehringer Mannheim) produced in bacteria are biologically active. Therefore, the glycosylated receptor probe should interact properly with its ligand synthesized by *E. coli* in a phase plaque during the screening.

In addition to using the Ap-tagged ALK-7 probe to screen for ALK-7 ligand *in vitro*, the probe can also be used in histological staining on mammalian brain section to localize expression of the ligand. Determination of the loci of expression of the ALK-7 ligand will allow for biochemical purification of the ligand from that tissue cell source further for analysis.

#### ***Functional Screening of the ALK-7 Ligand***

An alternative approach to isolate the ALK-7 ligand is to utilize a functional screening approach. Full length cDNA of ALK-7 is cloned into an expression vector pMEX under a MMLV LTR promoter. The ALK-7 expression vector is co-transfected into NIH/3T3 cells together with pSV2Hygro containing a hygromycin  $\beta$ -phosphotransferase gene which confers hygromycin resistance (Gritz & Davies, *Gene* 25:179-188 (1983)). The transfected cells are selected

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with hygromycin B at a concentration of 350 µg/ml. The resistant clones are grown in 12-well plates and screened for ALK-7 expression with anti-ALK-7 antibody by Western blot analysis.

5           The vector system developed by Miki *et al.*, *Gene* 83:137-146 (1989) is used to construct a directional eukaryotic cDNA library from rat brain mRNA. The vector has a MMLV LTR promoter for the expression of cDNA inserts and a SV40 early promoter-driven Neo gene as a selectable marker. In addition, this vector contains a pBR322 replication origin, and the cDNA inserts of interest can be obtained easily by *Not I* digestion of crude Lambda DNA preparations and  
10           ligation followed by transfection of bacterial cells. The cDNA library is constructed as described in detail by Miki *et al.*, *Gene* 83:137-146 (1989).

          The cDNA library is transfected into ALK-7-expressing NIH/3T3 mouse embryo fibroblasts. Foci from transfected cells are isolated and tested for Neo resistance to eliminate the background transformation in NIH/3T3 cells.  
15           Genomic DNA from each Neo-resistant transformant is cleaved by *Not I* which releases the plasmid. Digested DNA is ligated under diluted conditions and used to transform competent bacteria. Plasmid DNA from each focus is purified and transfected in NIH/3T3 cells with or without ALK-7 expression. The transformation by the ALK-7 ligand but not other proteins is expected to be  
20           dependent on the presence of ALK-7 expression. Clones are then further analyzed by sequencing, the encoded protein purified and assayed for ALK-7 binding.

### ***Example 3***

#### ***ALK-7 Protein***

25           An ALK-7 cDNA fragment was tagged with a hemagglutinating (HA) epitope in the C-terminus and was subcloned into pBS KS (Stratagene). The plasmid was used for *in vitro* translation with a kit of reagents from Promega following manufacturer's specifications. <sup>35</sup>S-Cys labeled products were

immunoprecipitated with monoclonal antibody anti-HA (12CA5), and separated by SDS/PAGE followed by autoradiography (Figure 2, left lane). ALK-7 protein consists of a predominant 55K band, additional bands were generated from internal Met codons.

5 ALK-7 HA-tagged cDNA was expressed from a pRc/MMV expression vector (Invitrogen) in COS cells. Transfected cells were labeled with  $^{125}\text{I}$ , lysed, immunoprecipitated with 12CA5 antibody, and analyzed by SDS/PAGE and autoradiography (Figure 2, right lane). A protein of 55K of the predicted size of  
10 ALK-7 was detected after  $^{125}\text{I}$  labeling of proteins in the surface of transfected cells.

#### ***Example 4***

##### ***ALK-7 mRNA Expression***

15 ALK-7 mRNA expression was studied by RNase protection analysis (RPA) in different regions of the adult rat brain (Figure 3, upper panel) and in developing peripheral tissues (Figure 3, lower panel). ALK-7 mRNA is preferentially restricted to the central nervous system. Predominant expression was observed in the adult cerebellum, but also in hippocampus, mesencephalon, pons, medula, colliculi and thalamus. COS cells did not express ALK-7 mRNA. Low ALK-7 mRNA expression was observed in 2 week-old, but not adult, rat  
20 ovary and in adult kidney. Also moderate ALK-7 mRNA expression was detected in the post-natal day 1 (P1) superior cervical ganglion (SCG).

25 ALK-7 mRNA expression analyzed by RPA during cerebellar development showed increased expression during cerebellum maturation, with highest levels in the adult cerebellum (Figure 4, left panel). A model of hypothyroidism was induced in new born rats with propylthiouracil (PTU), which delays development of granule and Purkinje cells in the cerebellum. The treatment also delayed the developmental expression of ALK-7 mRNA, indicating a role for ALK-7 in the maturation of different neuronal cell types in

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the cerebellum (Figure 4, middle panel). *In vitro*, ALK-7 mRNA was readily detected in cultures of granule cells (Figure 4, right panel).

5 A section through the adult cerebellum is shown in Figure 5, illustrating ALK-7 mRNA expression in Purkinje cells (arrowheads) and granule cells (arrow). The right panel shows ALK-7 mRNA expression in motoneurons of the facial motor nucleus (arrows).

\* \* \* \* \*

All publications mentioned hereinabove are hereby incorporated in their entirety by reference.

10 While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention and appended claims.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: IBÁÑEZ, CARLOS F.
- (ii) TITLE OF INVENTION: A Novel Serine Threonine Kinase Receptor
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Sterne, Kessler, Goldstein & Fox
  - (B) STREET: 1100 New York Avenue, Suite 600
  - (C) CITY: Washington
  - (D) STATE: DC
  - (E) COUNTRY: USA
  - (F) ZIP: 20005
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: (To Be Assigned)
  - (B) FILING DATE: Herewith
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 08/325,956
  - (B) FILING DATE: 20-OCT-1994
- (viii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 08/341,916
  - (B) FILING DATE: 15-NOV-1994
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Goldstein, Jorge A.
  - (B) REGISTRATION NUMBER: 29,021
  - (C) REFERENCE/DOCKET NUMBER: 1459.023PC02
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (202)371-2600
  - (B) TELEFAX: (202)371-2540

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1482 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: both
  - (D) TOPOLOGY: both
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 1..1482

- 54 -

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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1 5 10 15	
GCA CTG GCC TCC GAC CTT GCG GCA GGA CTG AAG TGT GTG TGT CTT TTG	96
Ala Leu Ala Ser Asp Leu Ala Ala Gly Leu Lys Cys Val Cys Leu Leu	
20 25 30	
TGT GAT TCC TCA AAC TTT ACC TGC CAA ACC GAA GGA GCA TGC TGG GCC	144
Cys Asp Ser Ser Asn Phe Thr Cys Gln Thr Glu Gly Ala Cys Trp Ala	
35 40 45	
TCT GTC ATG CTA ACC AAC GGG AAA GAA CAG GTG ATC AAA TCG TGC GTG	192
Ser Val Met Leu Thr Asn Gly Lys Glu Gln Val Ile Lys Ser Cys Val	
50 55 60	
TCC CTC CCG GAA CTA AAT GCT CAG GTC TTC TGT CAC AGT TCC AAC AAC	240
Ser Leu Pro Glu Leu Asn Ala Gln Val Phe Cys His Ser Ser Asn Asn	
65 70 75 80	
GTG ACC AAG ACC GAA TGT TGC TTC ACA GAC TTC TGC AAC AAC ATC ACT	288
Val Thr Lys Thr Glu Cys Cys Phe Thr Asp Phe Cys Asn Asn Ile Thr	
85 90 95	
CTG CAC CTT CCC ACA GCA TCT CCA GAT GCC CCT AGA CTT GGC CCC ACA	336
Leu His Leu Pro Thr Ala Ser Pro Asp Ala Pro Arg Leu Gly Pro Thr	
100 105 110	
GAG CTG ACA GTT GTT ATC ACT GTA CCT GTT TGC CTC CTG TCC ATC GCA	384
Glu Leu Thr Val Val Ile Thr Val Pro Val Cys Leu Leu Ser Ile Ala	
115 120 125	
GCC ATG CTA ACG ATA TGG GCC TGC CAG GAC CGC CAG TGC ACA TAC AGG	432
Ala Met Leu Thr Ile Trp Ala Cys Gln Asp Arg Gln Cys Thr Tyr Arg	
130 135 140	
AAG ACC AAG AGA CAC AAT GTG GAG GAA CCA CTG GCA GAG TAC AGC CTT	480
Lys Thr Lys Arg His Asn Val Glu Glu Pro Leu Ala Glu Tyr Ser Leu	
145 150 155 160	
GTC AAT GCT GGA AAA ACC CTC AAA GAT CTG ATT TAT GAT GCC ACT GCC	528
Val Asn Ala Gly Lys Thr Leu Lys Asp Leu Ile Tyr Asp Ala Thr Ala	
165 170 175	
TCG GGC TCA GGA TCT GGC CTG CCT CTT TTG GTT CAA AGA ACC ATC GCA	576
Ser Gly Ser Gly Ser Gly Leu Pro Leu Leu Val Gln Arg Thr Ile Ala	
180 185 190	
AGG ACA ATT GTA CTT CAA GAA ATC GTA GGA AAA GGT CGG TTT GGG GAA	624
Arg Thr Ile Val Leu Gln Glu Ile Val Gly Lys Gly Arg Phe Gly Glu	
195 200 205	
GTG TGG CAC GGA AGA TGG TGT GGA GAA GAT GTG GCT GTG AAA ATA TTC	672
Val Trp His Gly Arg Trp Cys Gly Glu Asp Val Ala Val Lys Ile Phe	
210 215 220	

- 55 -

TCC TCC AGA GAT GAG AGA TCT TGG TTC CGT GAG GCA GAA ATT TAT CAG Ser Ser Arg Asp Glu Arg Ser Trp Phe Arg Glu Ala Glu Ile Tyr Gln 225 230 235 240	720
ACG GTA ATG CTG AGA CAT GAG AAT ATT CTC GGT TTC ATC GCG GCC GAC Thr Val Met Leu Arg His Glu Asn Ile Leu Gly Phe Ile Ala Ala Asp 245 250 255	768
AAC AAA GAT AAT GGA ACC TGG ACT CAG CTT TGG CTT GTG TCA GAG TAT Asn Lys Asp Asn Gly Thr Trp Thr Gln Leu Trp Leu Val Ser Glu Tyr 260 265 270	816
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CAC CTA CAC ATG GAG ATC GTG GGC ACT CAA GGT AAG CCT GCT ATT GCT His Leu His Met Glu Ile Val Gly Thr Gln Gly Lys Pro Ala Ile Ala 305 310 315 320	960
CAC CGA GAT ATA AAG TCA AAG AAT ATC TTA GTC AAA AAG TGT GAC ACT His Arg Asp Ile Lys Ser Lys Asn Ile Leu Val Lys Lys Cys Asp Thr 325 330 335	1008
TGT GCC ATA GCT GAC TTA GGG CTG GCT GTG AAA CAT GAT TCT ATC ATG Cys Ala Ile Ala Asp Leu Gly Leu Ala Val Lys His Asp Ser Ile Met 340 345 350	1056
AAC ACT ATA GAT ATA CCC CAG AAT CCT AAA GTG GGA ACC AAG AGG TAT Asn Thr Ile Asp Ile Pro Gln Asn Pro Lys Val Gly Thr Lys Arg Tyr 355 360 365	1104
ATG GCT CCC GAA ATG CTT GAT GAT ACA ATG AAC GTC AAC ATC TTT GAG Met Ala Pro Glu Met Leu Asp Asp Thr Met Asn Val Asn Ile Phe Glu 370 375 380	1152
TCC TTC AAG CGA GCT GAC ATC TAT TCG GTG GGG CTG GTT TAC TGG GAA Ser Phe Lys Arg Ala Asp Ile Tyr Ser Val Gly Leu Val Tyr Trp Glu 385 390 395 400	1200
ATA GCT CGA AGG TGT TCA GTT GGA GGA CTT GTT GAA GAG TAC CAG TTG Ile Ala Arg Arg Cys Ser Val Gly Gly Leu Val Glu Glu Tyr Gln Leu 405 410 415	1248
CCT TAT TAT GAC ATG GTG CCT TCA GAT CCT TCC ATA GAG GAA ATG AGG Pro Tyr Tyr Asp Met Val Pro Ser Asp Pro Ser Ile Glu Glu Met Arg 420 425 430	1296
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CAA AGC TGT GAG GCG CTC CGG GTC ATG GGA AGA ATA ATG CGT GAG TGC Gln Ser Cys Glu Ala Leu Arg Val Met Gly Arg Ile Met Arg Glu Cys 450 455 460	1392

- 56 -

TGG TAT GCC AAC GGG GCA GCT CGC CTG ACC GCC CTG CGC GTG AAG AAG 1440  
 Trp Tyr Ala Asn Gly Ala Ala Arg Leu Thr Ala Leu Arg Val Lys Lys  
 465 470 475 480

ACC ATT TCT CAG CTG TGT GTC AAG GAA GAC TGT AAG GCC TAA 1482  
 Thr Ile Ser Gln Leu Cys Val Lys Glu Asp Cys Lys Ala  
 485 490

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 493 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Thr Pro Ala Ser Arg Ser Ala Leu Ser Leu Ala Leu Leu Leu Val  
 1 5 10 15  
 Ala Leu Ala Ser Asp Leu Ala Ala Gly Leu Lys Cys Val Cys Leu Leu  
 20 25 30  
 Cys Asp Ser Ser Asn Phe Thr Cys Gln Thr Glu Gly Ala Cys Trp Ala  
 35 40 45  
 Ser Val Met Leu Thr Asn Gly Lys Glu Gln Val Ile Lys Ser Cys Val  
 50 55 60  
 Ser Leu Pro Glu Leu Asn Ala Gln Val Phe Cys His Ser Ser Asn Asn  
 65 70 75 80  
 Val Thr Lys Thr Glu Cys Cys Phe Thr Asp Phe Cys Asn Asn Ile Thr  
 85 90 95  
 Leu His Leu Pro Thr Ala Ser Pro Asp Ala Pro Arg Leu Gly Pro Thr  
 100 105 110  
 Glu Leu Thr Val Val Ile Thr Val Pro Val Cys Leu Leu Ser Ile Ala  
 115 120 125  
 Ala Met Leu Thr Ile Trp Ala Cys Gln Asp Arg Gln Cys Thr Tyr Arg  
 130 135 140  
 Lys Thr Lys Arg His Asn Val Glu Glu Pro Leu Ala Glu Tyr Ser Leu  
 145 150 155 160  
 Val Asn Ala Gly Lys Thr Leu Lys Asp Leu Ile Tyr Asp Ala Thr Ala  
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 Ser Gly Ser Gly Ser Gly Leu Pro Leu Leu Val Gln Arg Thr Ile Ala  
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 195 200 205

- 57 -

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 260 265 270  
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 305 310 315 320  
 His Arg Asp Ile Lys Ser Lys Asn Ile Leu Val Lys Lys Cys Asp Thr  
 325 330 335  
 Cys Ala Ile Ala Asp Leu Gly Leu Ala Val Lys His Asp Ser Ile Met  
 340 345 350  
 Asn Thr Ile Asp Ile Pro Gln Asn Pro Lys Val Gly Thr Lys Arg Tyr  
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 Met Ala Pro Glu Met Leu Asp Asp Thr Met Asn Val Asn Ile Phe Glu  
 370 375 380  
 Ser Phe Lys Arg Ala Asp Ile Tyr Ser Val Gly Leu Val Tyr Trp Glu  
 385 390 395 400  
 Ile Ala Arg Arg Cys Ser Val Gly Gly Leu Val Glu Glu Tyr Gln Leu  
 405 410 415  
 Pro Tyr Tyr Asp Met Val Pro Ser Asp Pro Ser Ile Glu Glu Met Arg  
 420 425 430  
 Lys Val Val Cys Asp Gln Lys Leu Arg Pro Asn Leu Pro Asn Gln Trp  
 435 440 445  
 Gln Ser Cys Glu Ala Leu Arg Val Met Gly Arg Ile Met Arg Glu Cys  
 450 455 460  
 Trp Tyr Ala Asn Gly Ala Ala Arg Leu Thr Ala Leu Arg Val Lys Lys  
 465 470 475 480  
 Thr Ile Ser Gln Leu Cys Val Lys Glu Asp Cys Lys Ala  
 485 490

- 58 -

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 25 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCGGATCCGT BGCHGTBAAR ATHTT

25

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Val Ala Val Lys Ile Phe  
1 5

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGGAATTCDG GDGCCATRTA

20

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 5 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Tyr Met Ala Pro Glu  
1 5

***What Is Claimed Is:***

1. An isolated nucleic acid molecule coding for a polypeptide comprising an amino acid sequence corresponding to a serine threonine kinase receptor, ALK-7.

2. The isolated nucleic acid molecule according to claim 1, wherein the molecule has the nucleic acid sequence set forth in SEQ ID NO:1.

3. The isolated nucleic acid molecule according to claim 1, wherein the molecule encodes the amino acid sequence set forth in SEQ ID NO:2.

4. A substantially pure polypeptide comprising an amino acid sequence corresponding to ALK-7.

5. The polypeptide according to claim 4, wherein the polypeptide comprises the amino acid sequence set forth in SEQ ID NO:2.

6. A nucleic acid probe for the detection of the presence of ALK-7 in a DNA sample from an individual comprising a nucleic acid molecule sufficient to specifically detect under stringent hybridization conditions the presence of the molecule according to claim 1 in said sample.

7. A method of detecting ALK-7 nucleic acid in a sample comprising:

- a) contacting said sample with the nucleic acid probe according to claim 6, under conditions such that hybridization occurs, and
- b) detecting the presence of said probe bound to ALK-7 nucleic acid.

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8. A kit for detecting the presence of ALK-7 nucleic acid in a sample comprising at least one container means having disposed therein the nucleic acid probe according to claim 6.

5 9. A recombinant nucleic acid molecule comprising, 5' to 3', a promoter effective to initiate transcription in a host cell and the isolated nucleic acid molecule according to claim 1.

10 10. A recombinant nucleic acid molecule comprising a vector and the isolated nucleic acid molecule according to claim 1.

11. A cell that contains the recombinant nucleic acid molecule according to any one of claims 9 or 10.

15 12. A non-human organism that contains the recombinant nucleic acid molecule according to any one of claims 9 or 10.

13. An antibody having binding affinity to the polypeptide of claim 4.

20 14. A method of detecting an ALK-7 polypeptide in a sample, comprising:

a) contacting said sample with an antibody according to claim 13, under conditions such that immunocomplexes form, and  
b) detecting the presence of said antibody bound to said polypeptide.  
25

15. A diagnostic kit comprising:

a) a first container means containing the antibody according to claim 13 and

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b) second container means containing a conjugate comprising a binding partner of said monoclonal antibody and a label.

5 16. A hybridoma which produces the monoclonal antibody according to claim 13.

17. A bioassay for assessing candidate drugs or ligands of the ALK-7 receptor comprising:

10 a) contacting a candidate drug or ligand with a cell producing the polypeptide according to claim 4; and

b) evaluating the biological activity mediated by said contact.

15 18. A method of treatment of a neurological disease in a mammal, comprising administering a therapeutically effective amount of the polypeptide of claim 4 in a gene delivery system to said mammal.

20 19. The method of claim 18, wherein said disease is selected from the group consisting of neurodegenerative diseases, neurodegenerative disorders and neurodegenerative injuries.

20. A ligand capable of binding to the polypeptide according to claim 4.

25 21. A method of treatment of neurological disease in a mammal, comprising administering a therapeutically effective amount of the ligand according to claim 21 to a mammal afflicted with said disease.

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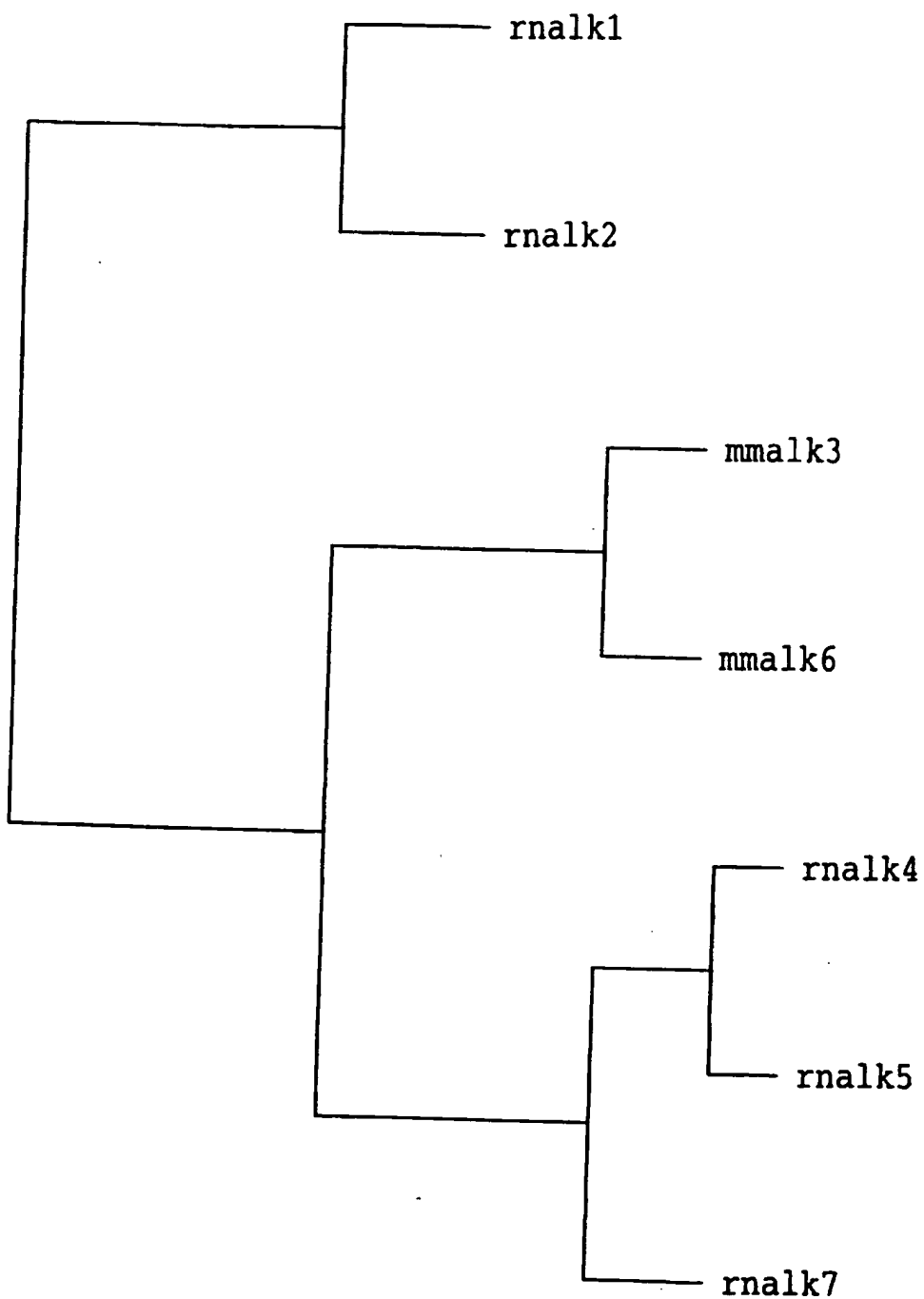


FIG.1

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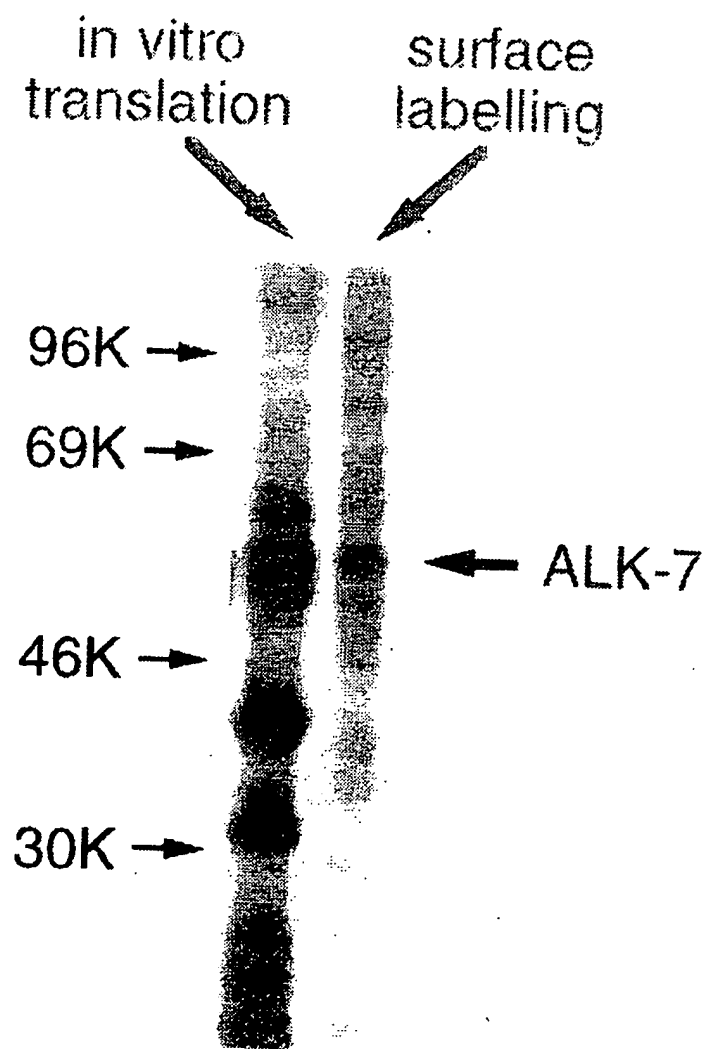


FIG.2

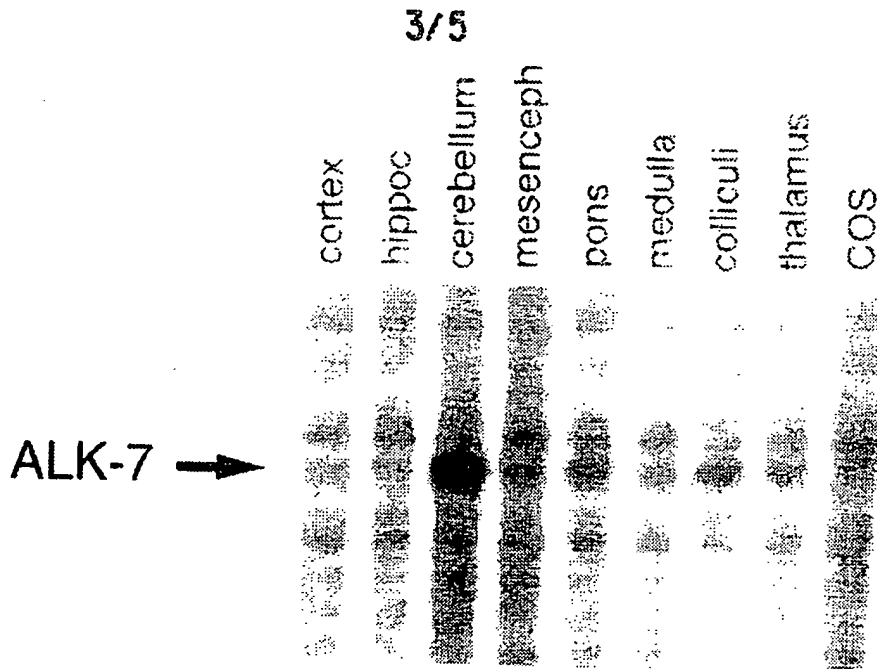


FIG.3A

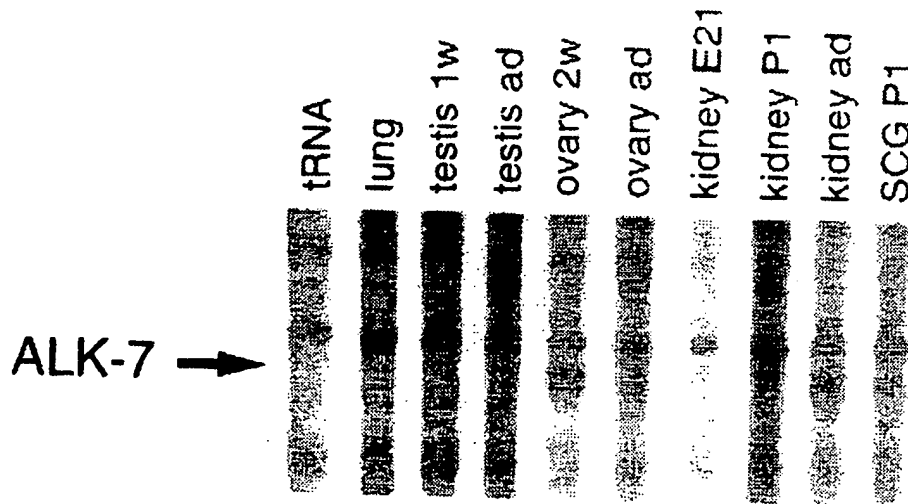
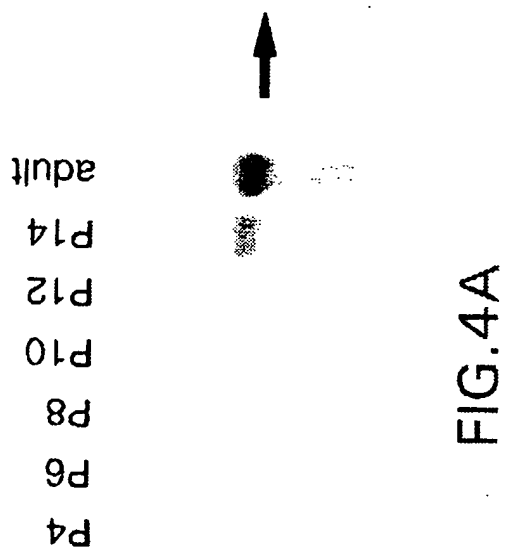
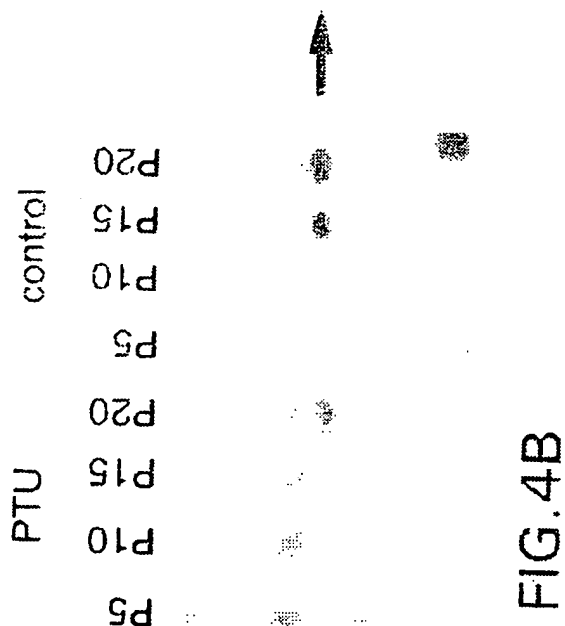
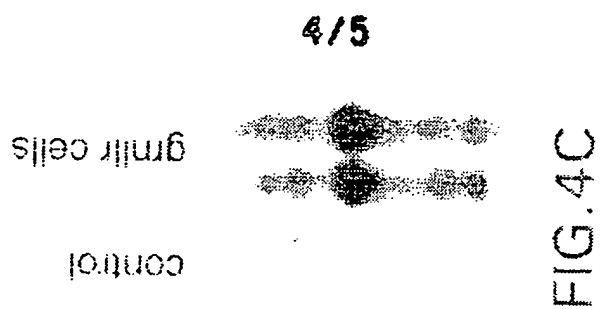


FIG.3B



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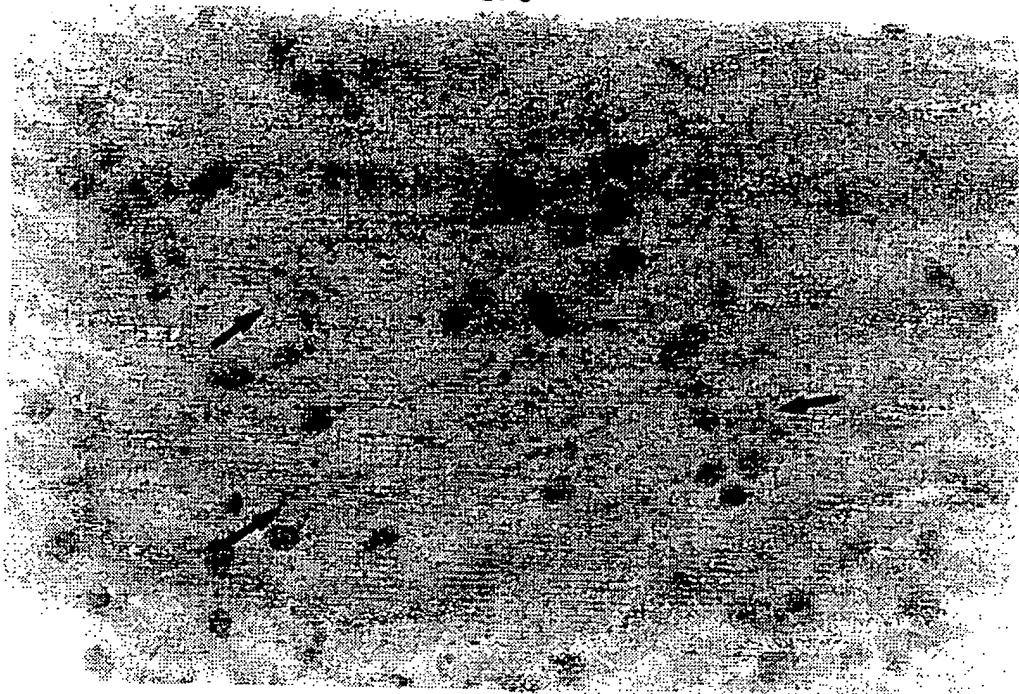


FIG. 5B

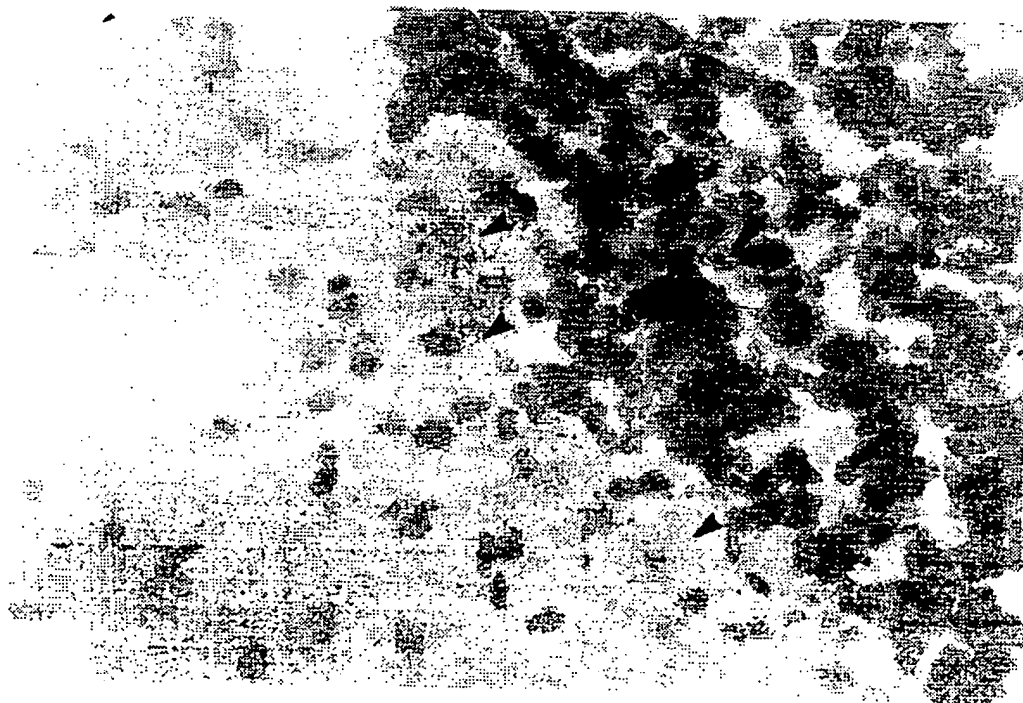


FIG. 5A

SUBSTITUTE SHEET (RULE 26)

## INTERNATIONAL SEARCH REPORT

Inter national Application No

PCT/IB 95/00899

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/71 C12N5/10 A61K38/17 G01N33/68  
 C07K16/18 C12N1/21 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	SOCIETY FOR NEUROSCIENCE ABSTRACTS, 21 (1-3): 1995. 1784., RYDEN M ET AL 'ALK-7, a novel brain specific serine-threonine kinase receptor' see the whole document	1-21
A	WO,A,94 11502 (LUDWIG INST CANCER RES ;MIYAZONO KOHEI (SE); DIJKE PETER TEN (SE);) 26 May 1994 see the whole document -/--	1-21

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principles or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*A\* document member of the same patent family

Date of the actual completion of the international search

19 February 1996

Date of mailing of the international search report

2 8. 02. 96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
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Authorized officer

Nauche, S

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 95/00899

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>SCIENCE, vol. 264, 1994 LANCASTER, PA US, pages 101-104, TEN DIJKE P;YAMASHITA H;ICHIJO H;FRANZEN P;LAIHO M;MIYAZONO K;HELDIN C-H; 'Characterization of type I receptors for transforming growth factor-beta and activin' see the whole document -----</p>	1-21

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 95/00899

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9411502	26-05-94	AU-B- 5432094	08-06-94
		CA-A- 2149441	26-05-94
		EP-A- 0677104	18-10-95
-----			